

# Isolation of Non-Cytopathic (NCP) VSV Mutants

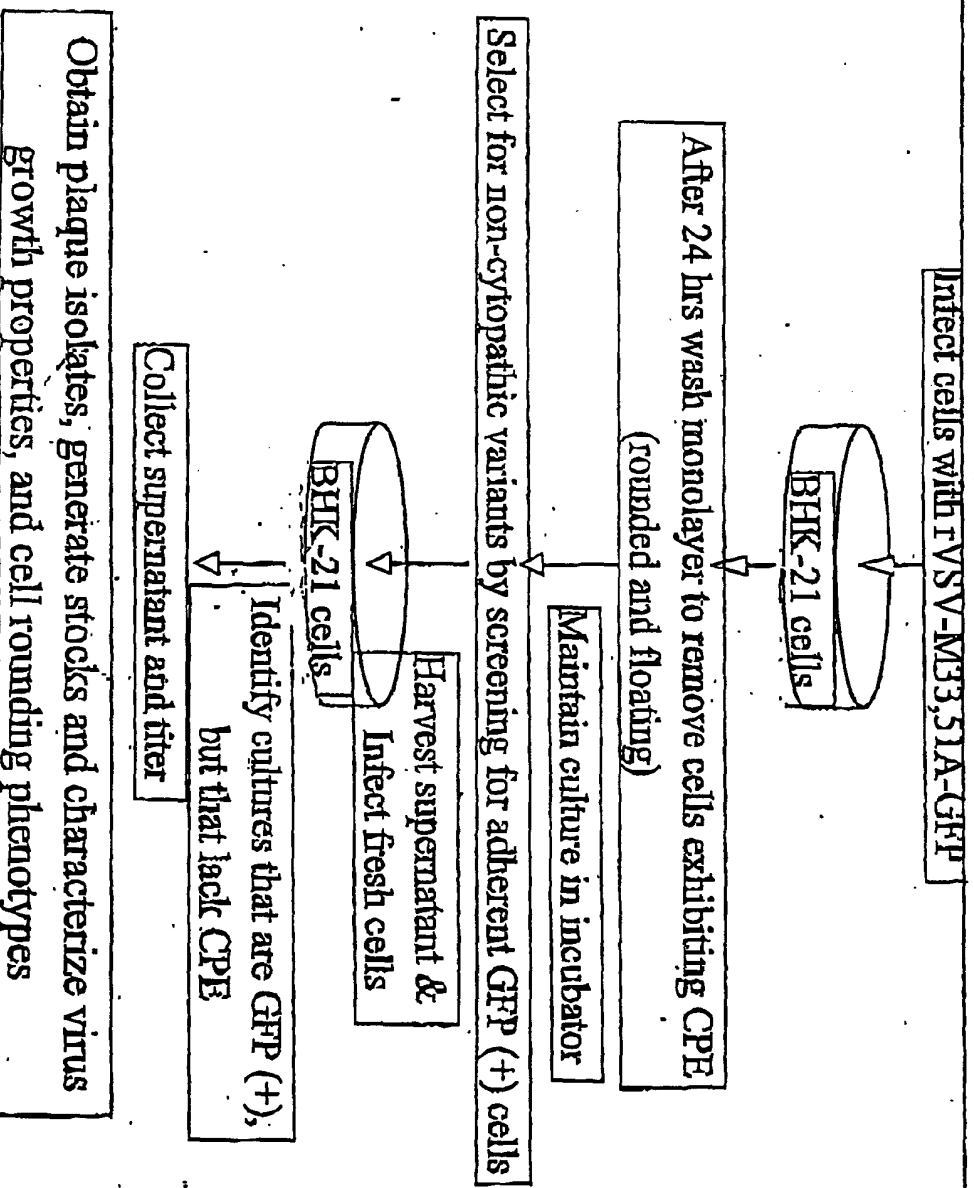
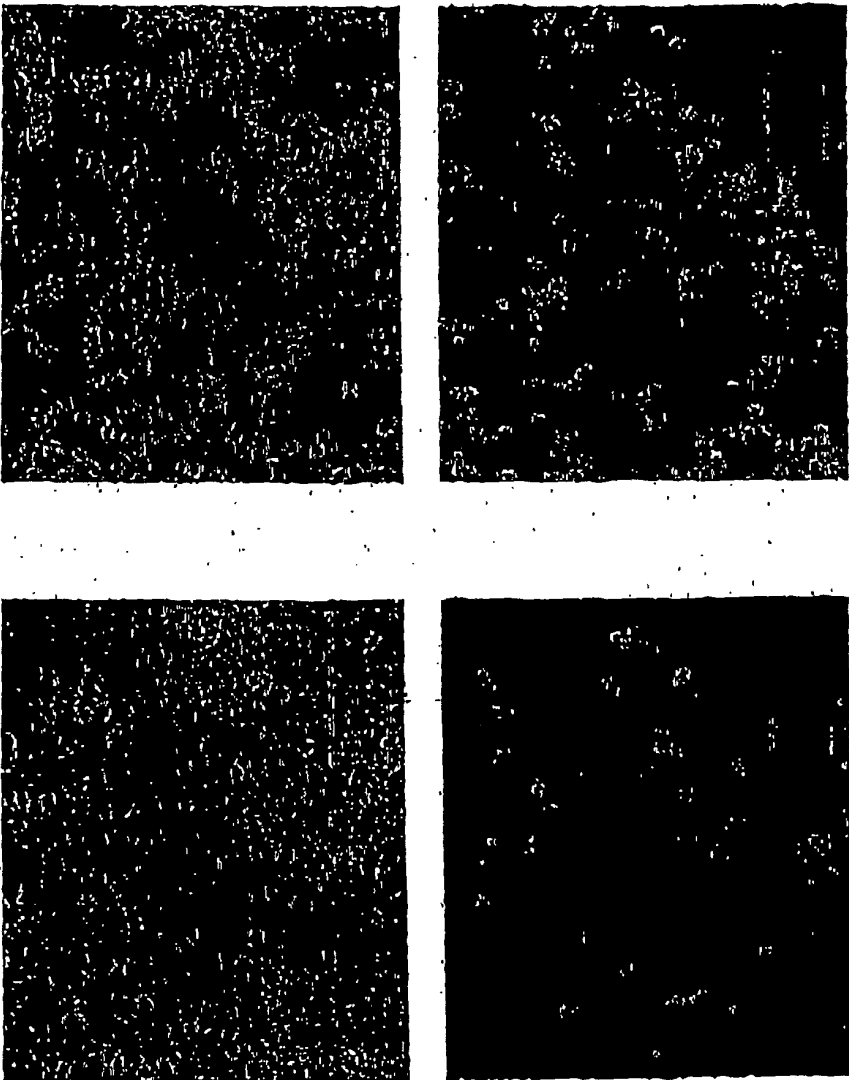


Figure 1

# Cell Rounding Phenotypes of M Protein Mutants of VSV



# Isolation and Sequencing of NCP-M Protein Mutant cDNAs

Purify NCP virus from culture supernatants of  
plaque purified NCP isolates



Isolate genomic RNA and perform RT-PCR using M gene specific primers



Subclone cDNAs into plasmid pBS-SK+



Sequence individual clones and identify mutations present in M<sub>NCP</sub> cDNAs



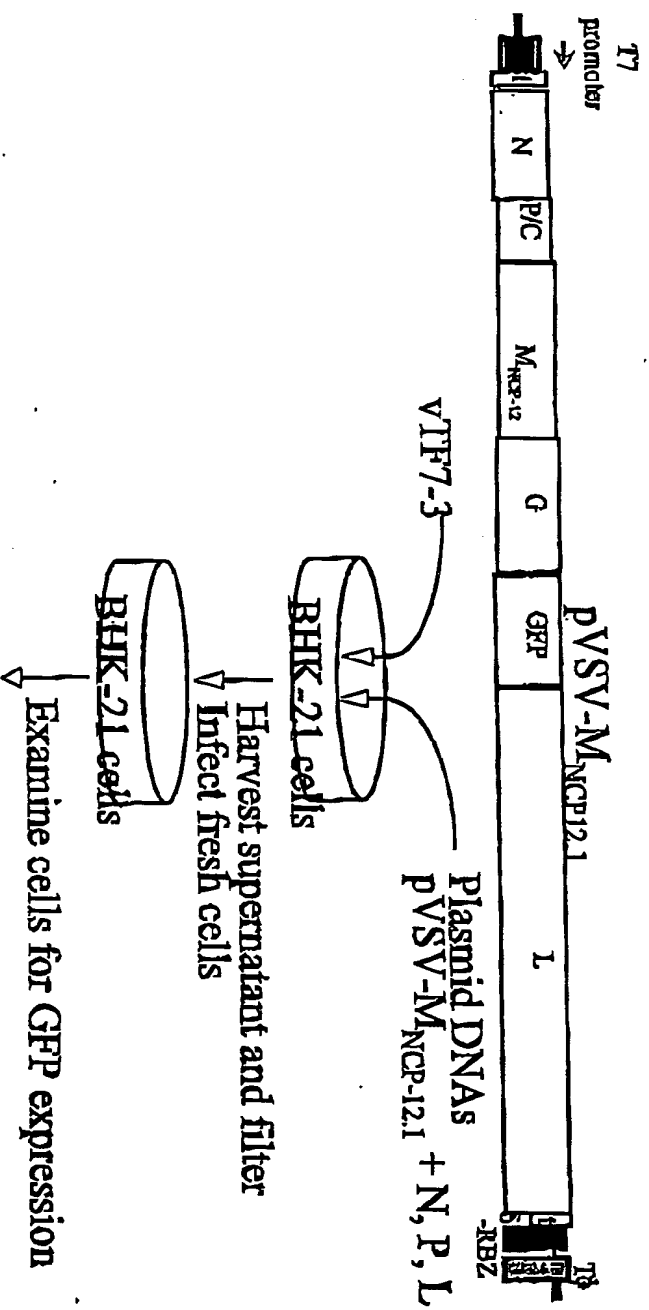
NCP mutations are M33A; M51A; T133A; & S226G

Designated M<sub>NCP12.1</sub>

Subclone M<sub>NCP12.1</sub> into pVSV-FL(+)-2

Figure 3

# Recovery of rVSV-M<sub>NCP12.1</sub>



Collect supernatant and titer

Obtain plaque isolates, generate stocks and characterize growth properties, and cell roundness

Figure 4

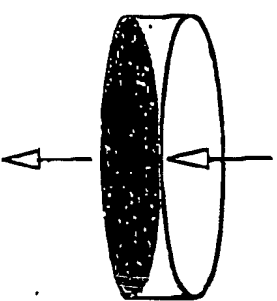
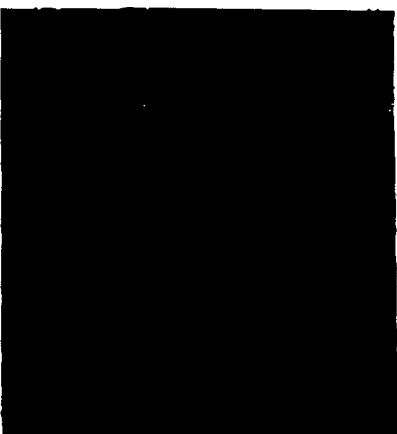
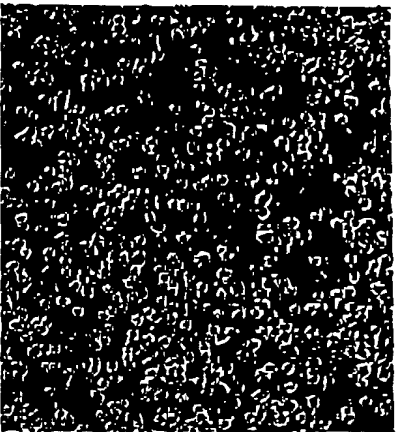
# rVSV/M<sub>NCP12.1</sub> is Defective in Cell Rounding

Phase Contrast

Anti-VSV N protein MAAb

Infect BHK-21 cells

wt rVSV



Examine @ 12 hrs p.i.

rVSV  
NCP-12.1

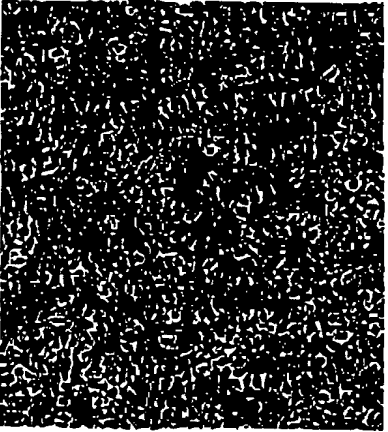


Figure 5

# Transient Expression of $M_{NCP-12.1}$

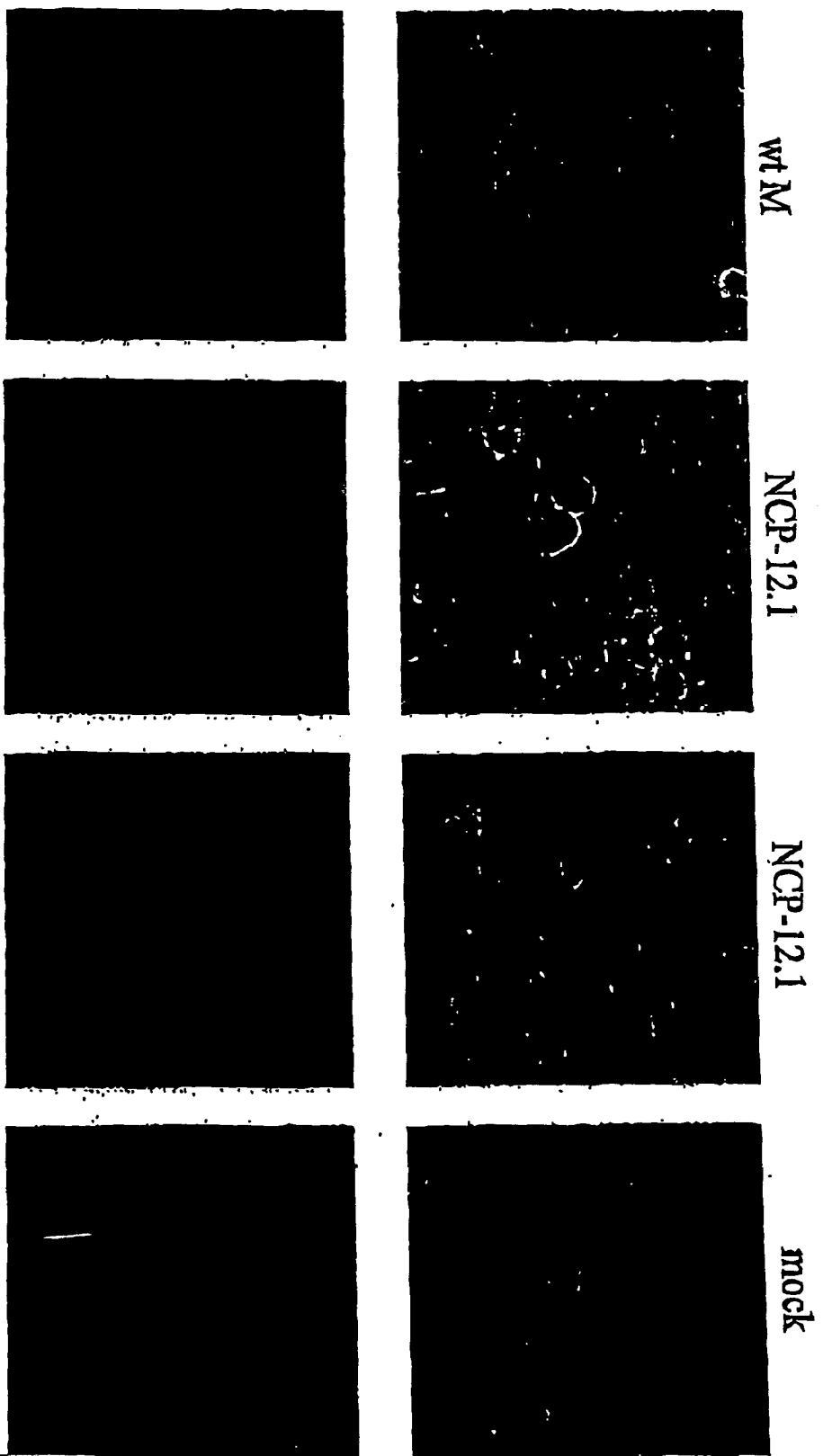


Figure 6

# rVSV/M<sub>NCP-12.1</sub> Infection of Different Cell Types

BHK @ 24 hr

CV-1 @ 24 hr

Hela @ 12 hr

Vero @ 24 hr

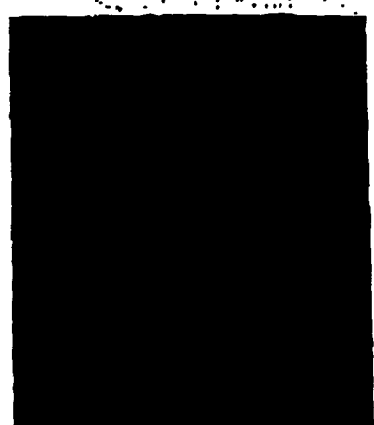
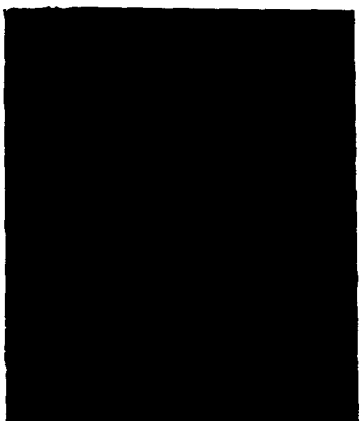


Figure 7

# Use of $M_{NCP-12.1}$ to Recover $\Delta M$ -VSV

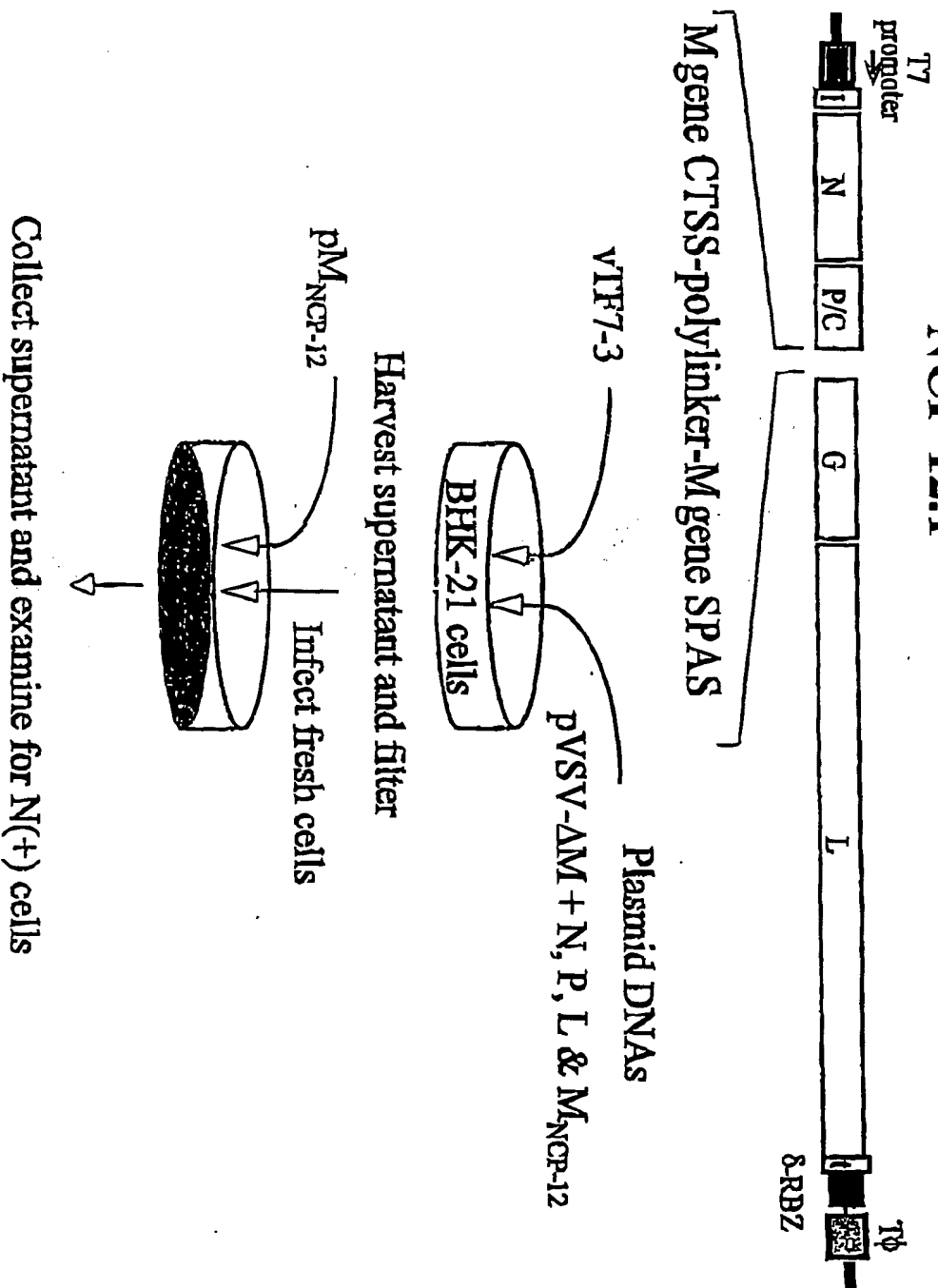


Figure 8



# Recovery of rVSV-ΔM

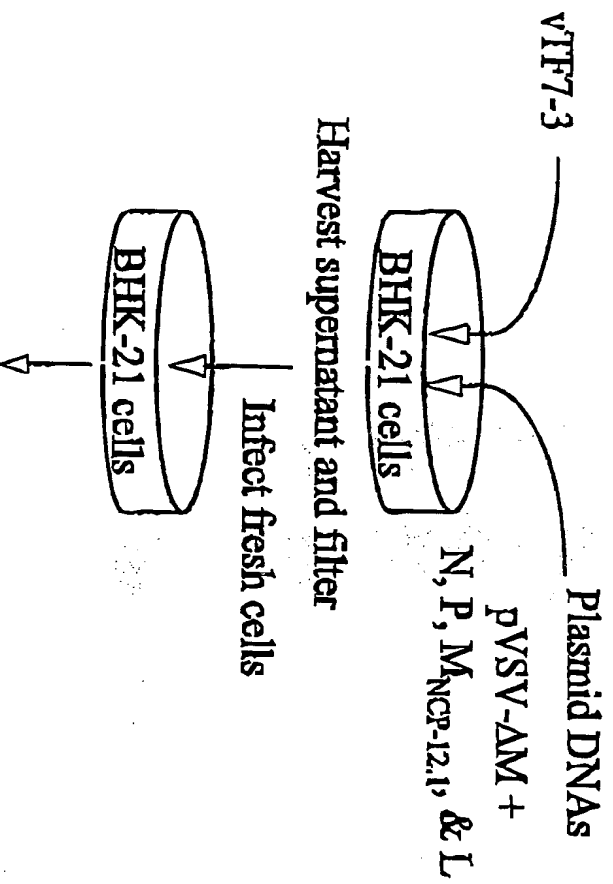
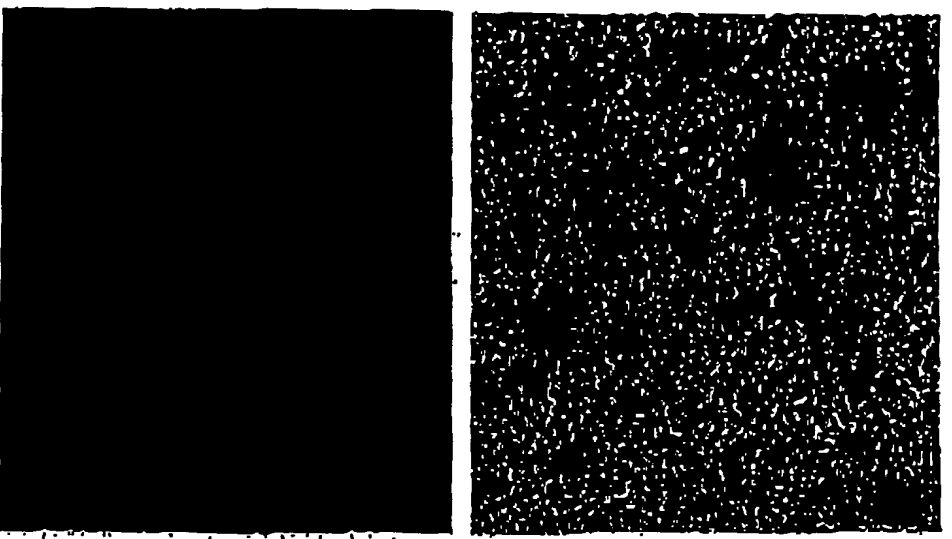


Figure 9



# Sample #176, 3 Days Post-infection

Infection of islet Prep #176 at day 3 post infection

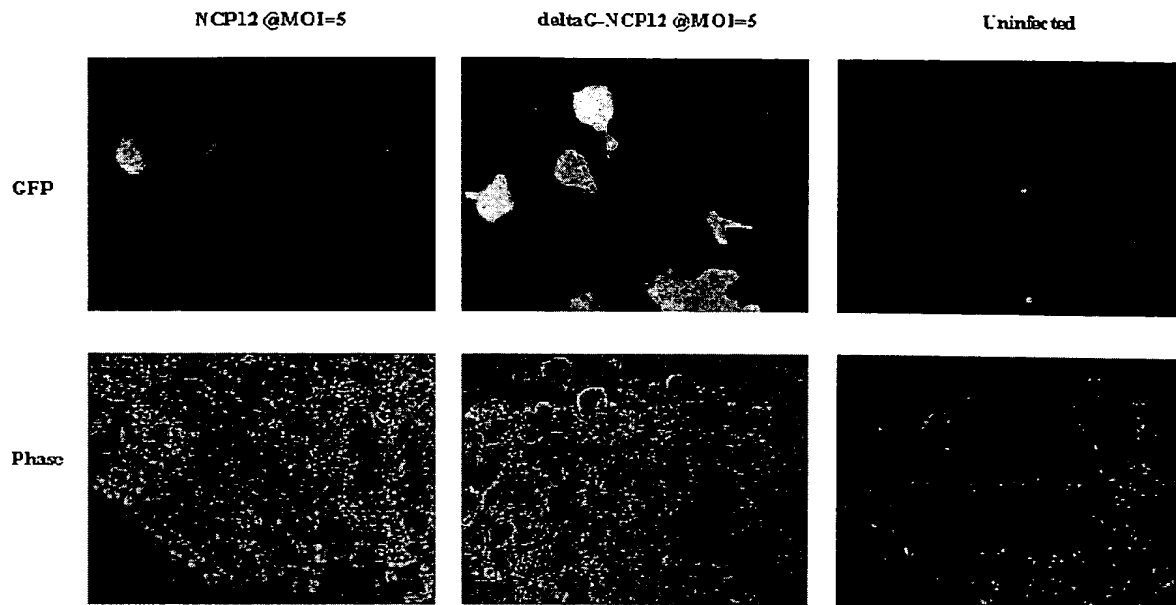


Figure 10

# Sample #163, 3 Days Post-infection

Infection of islet Prep#163 at day 3 post infection

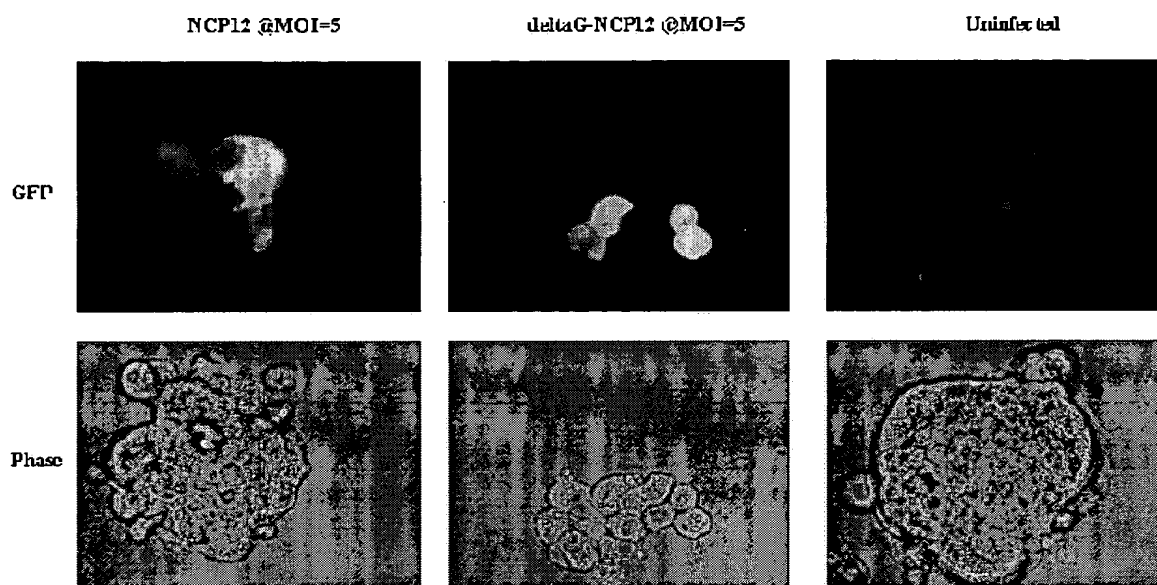


Figure 11

# Sample #176, 3 Days Post-infection

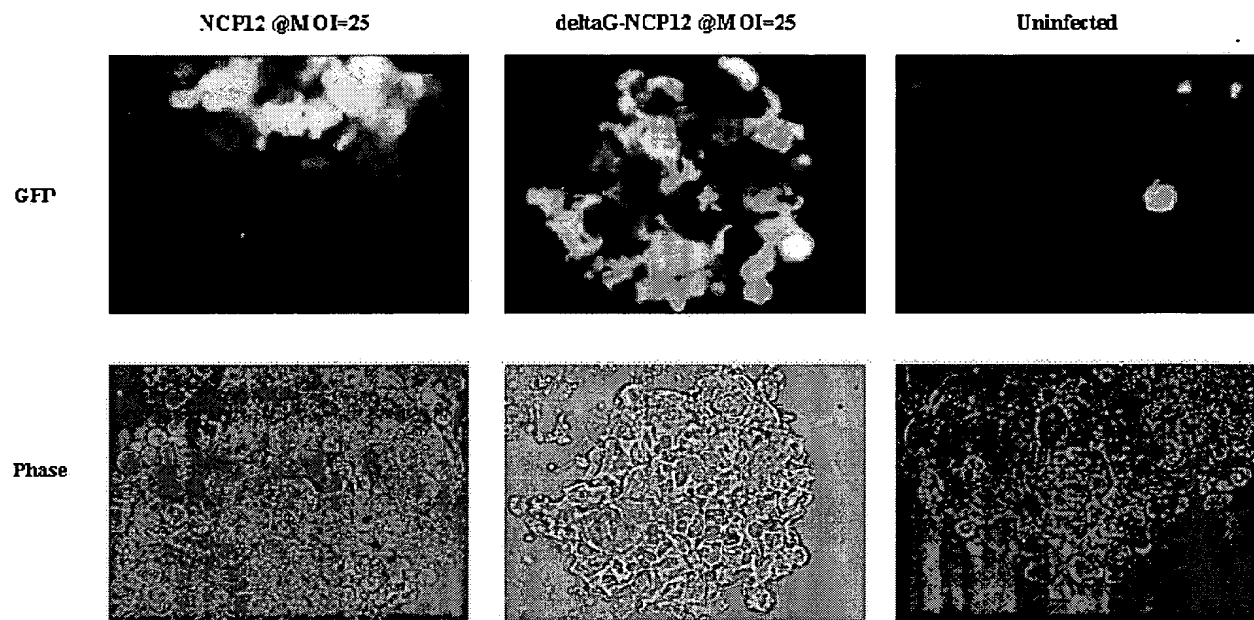


Figure 12

# Sample #163, 3 Days Post-infection

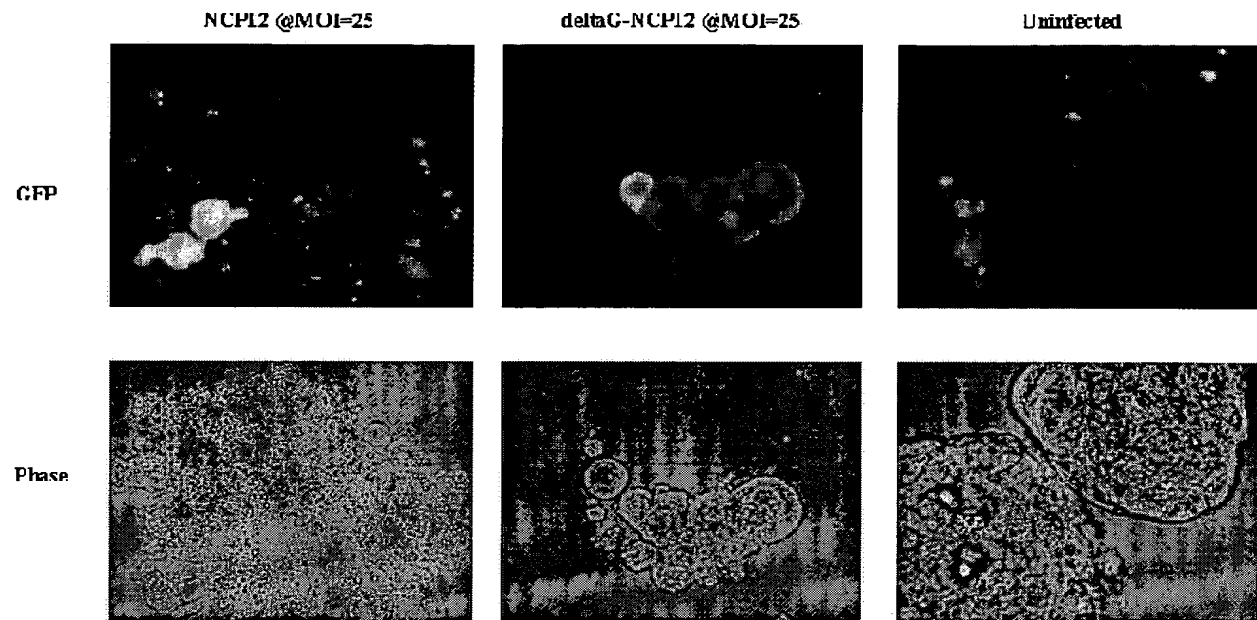


Figure 13

# Sample #176, 8 Days Post-infection

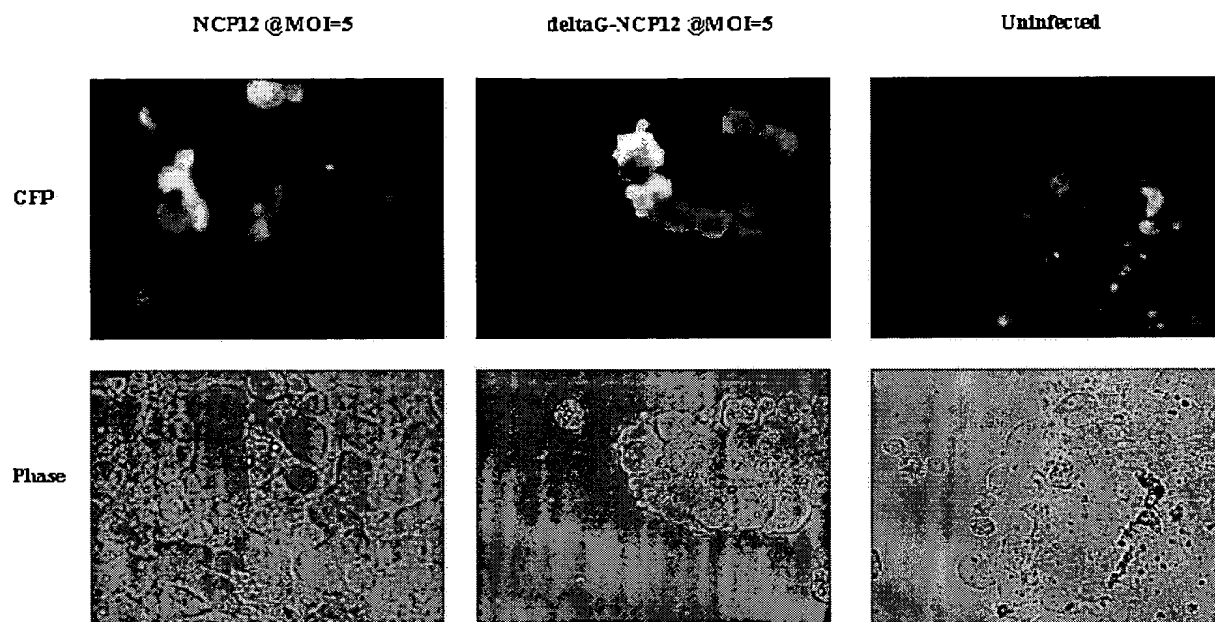


Figure 14

# Sample #176, 8 Days Post-infection

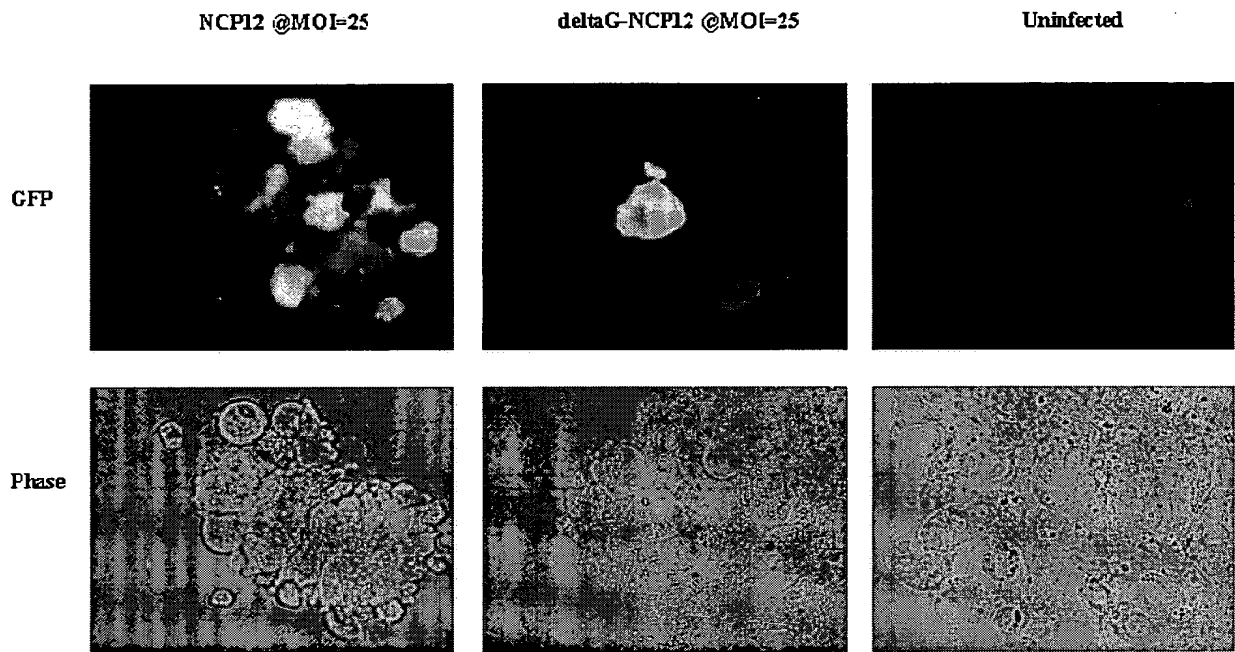


Figure 15

# Sample #163, 8 Days Post-infection

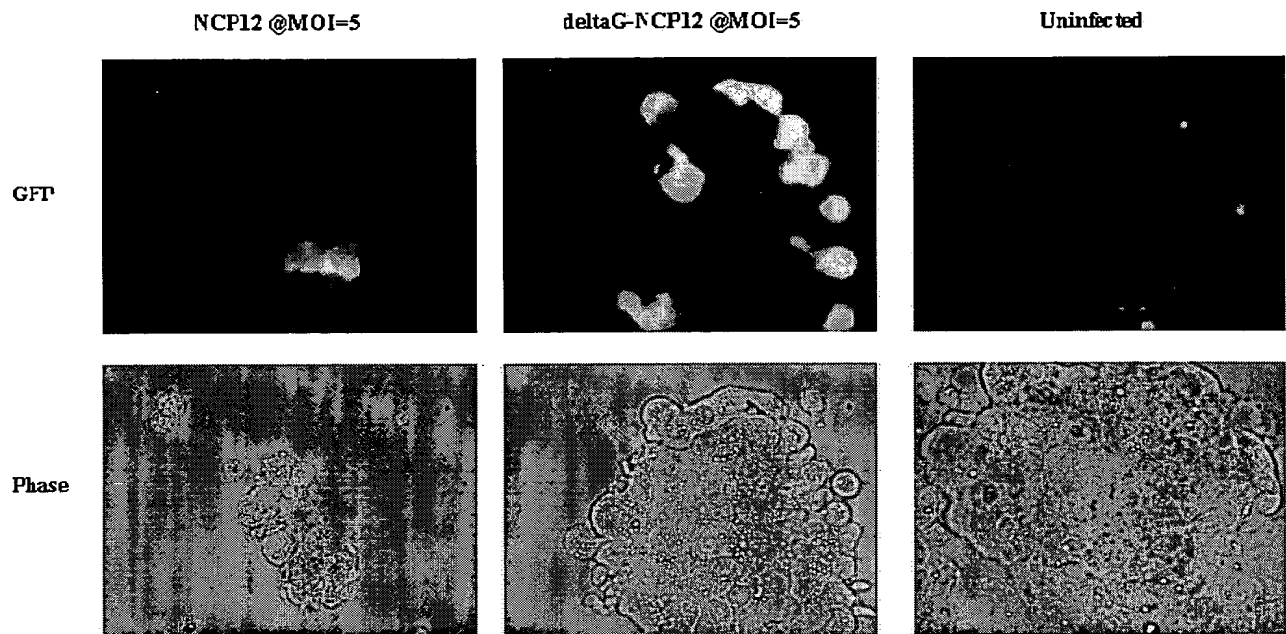


Figure 16



# Sample #163, 8 Days Post-infection

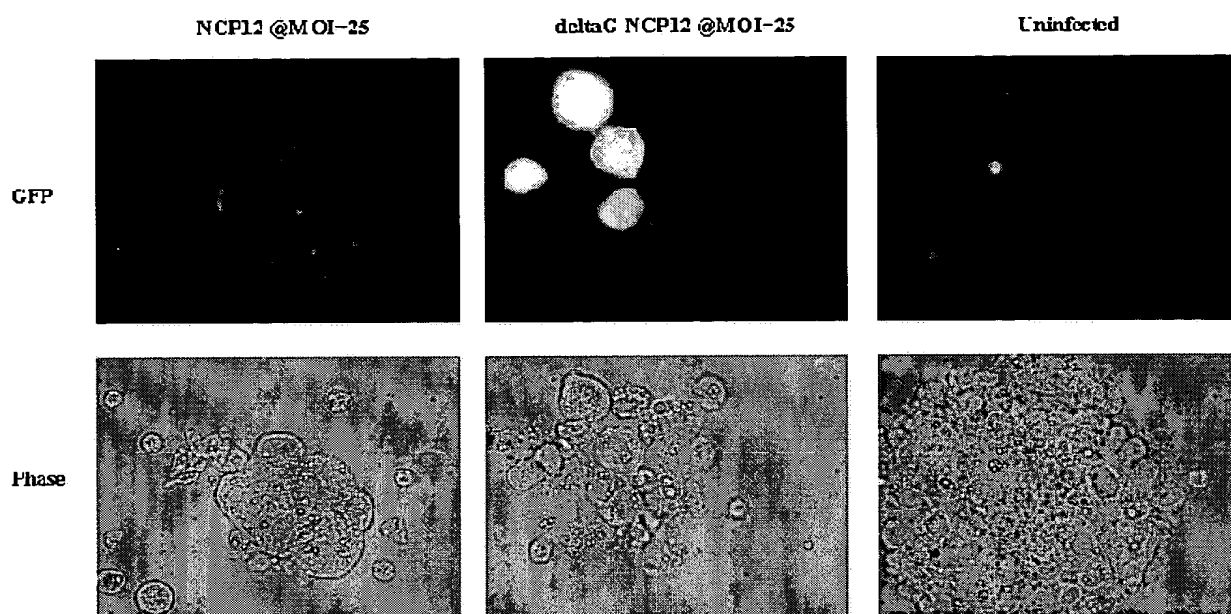


Figure 17

# Sample #176, 3 Days Post-infection

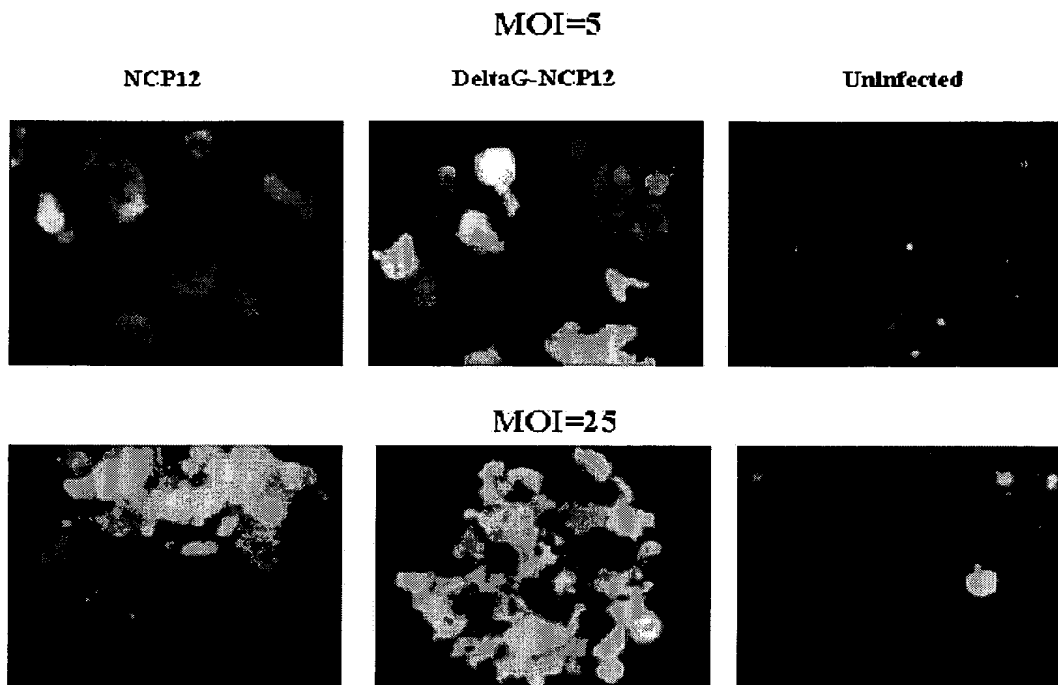


Figure 18

# Sample #176, 8 Days Post-infection

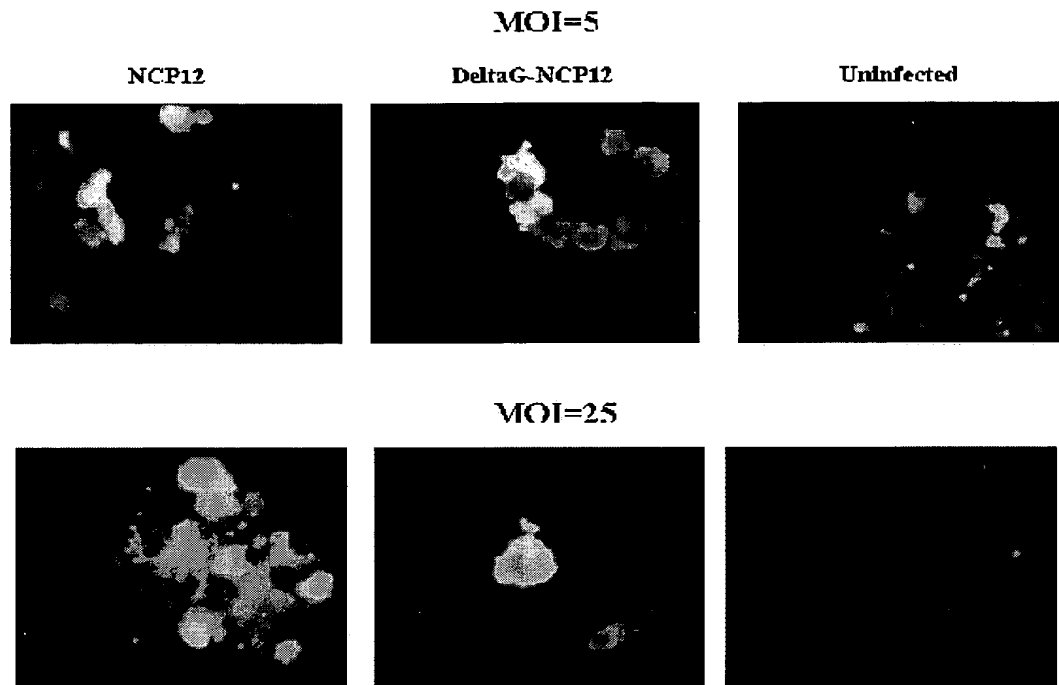


Figure 19

	San Juan	421	--FEEHIOBAASTLDD--	ESTLEFGDLSKNIITVEGSSS
	Orcay	421	--	--
	Cocal	421	--	--
	New Jersey	421	--	--
	Pty	421	--	--
	Chandipura	421	--	--
	Spring Virenia	421	ADIIIP	H---YDDNIREDDNDNENGNGLA VEVG

Domain A
Domain B

462

H452A(B-A)  
G456D(G-D)  
W457A(W1A)  
R458A(F-A)  
W461A(W2A)  
W457A,W461A(WW-AA)  
G456D,W457A(DA)  
W457A,R458A,W461A(AA)  
G456D,W457A,W461A(DA)

FHHPHI0DAA\$0LPDDE\$LFEGDTGL\$KNPIELVEGWFS\$WKSS  
 GTEV11  
 GTEV11  
 GTEV11-AA  
 AF440-N449  
 G413  
 G(AXB)  
 FHHPHI0DAA\$0LPDDE\$LFEGDTGL\$KNPIELVEGWFS\$WKSS  
 G49  
 FHHPHI0DAA\$0LPDDE\$LFEGDTGL\$KNPIELVEGWFS\$WKSS  
 G10DAR  
 FHHPHI0DAA\$0LPDDE\$LFEGDTGL\$KNPIELVEGWFS\$WKSS  
 G69-10DAR  
 FHHPHI0DAA\$0LPDDE\$LFEGDTGL\$KNPIELVEGWFS\$WKSS  
 G(9)2BC

Figure 21

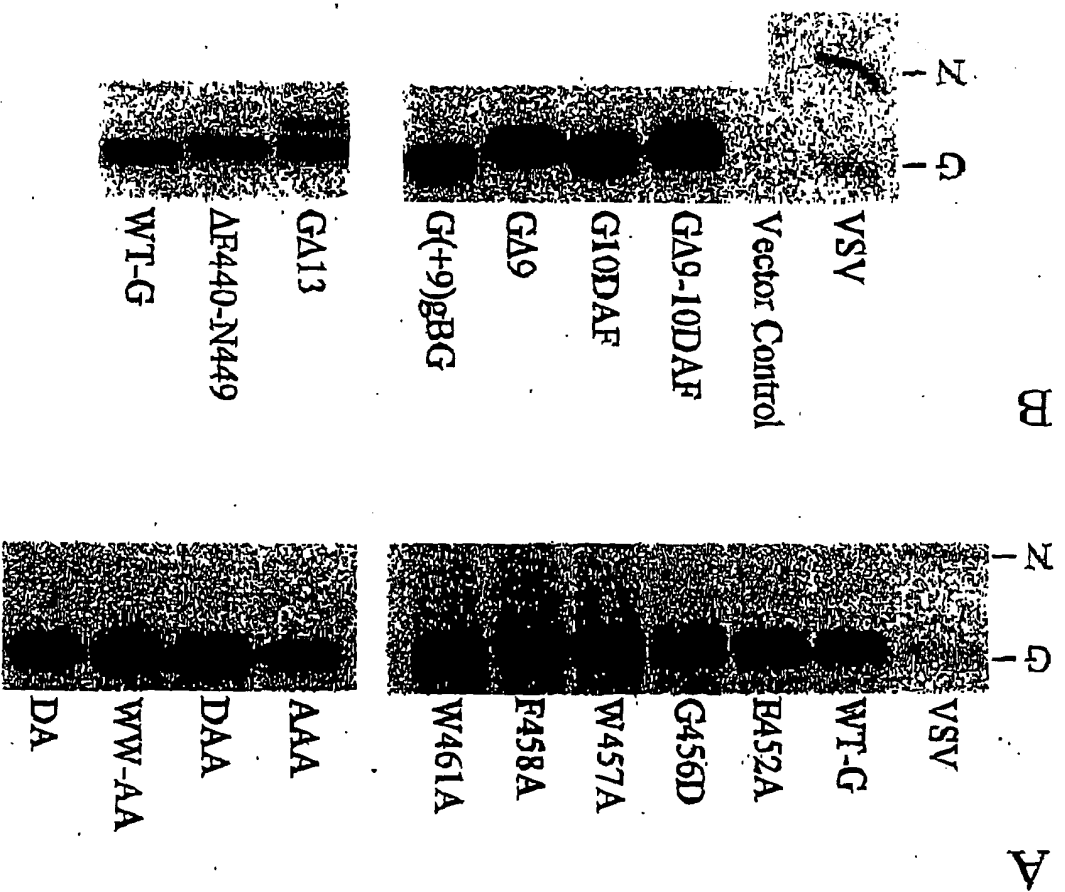
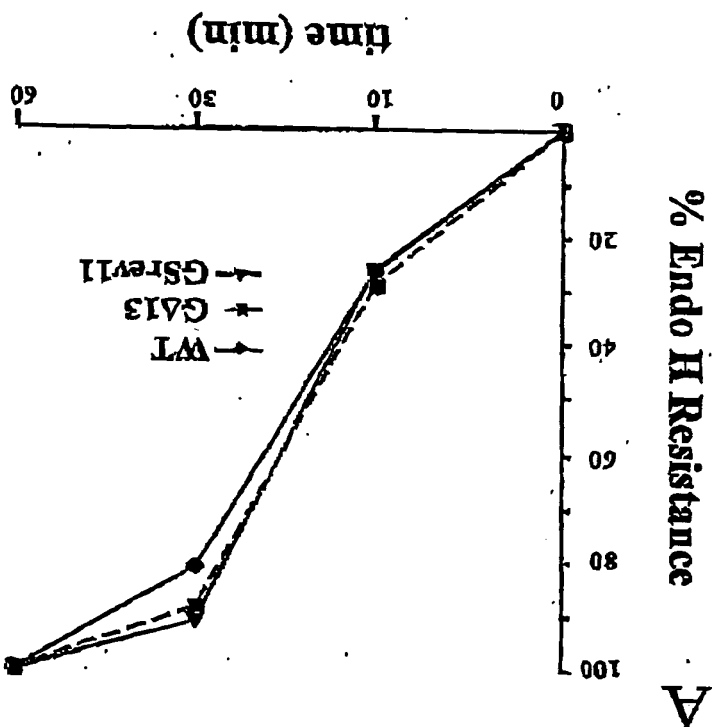
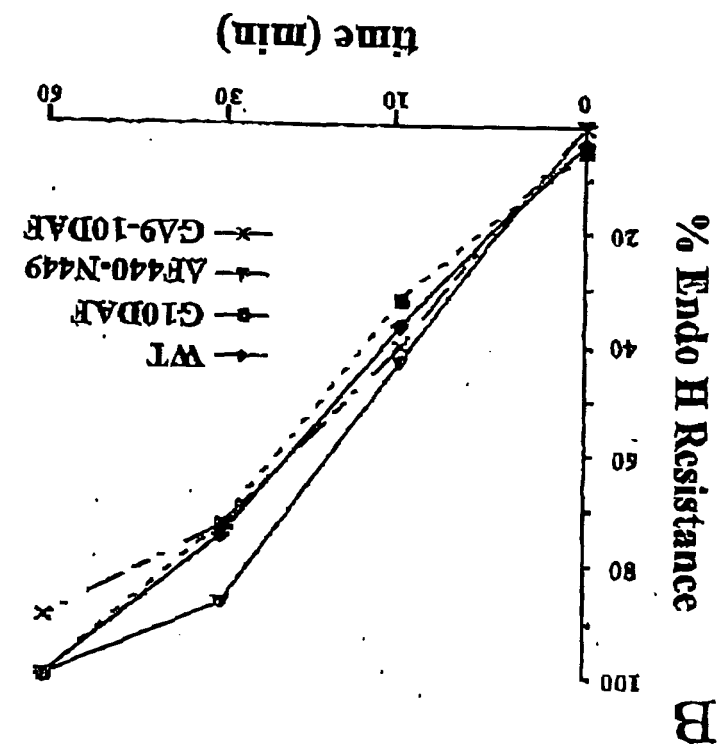
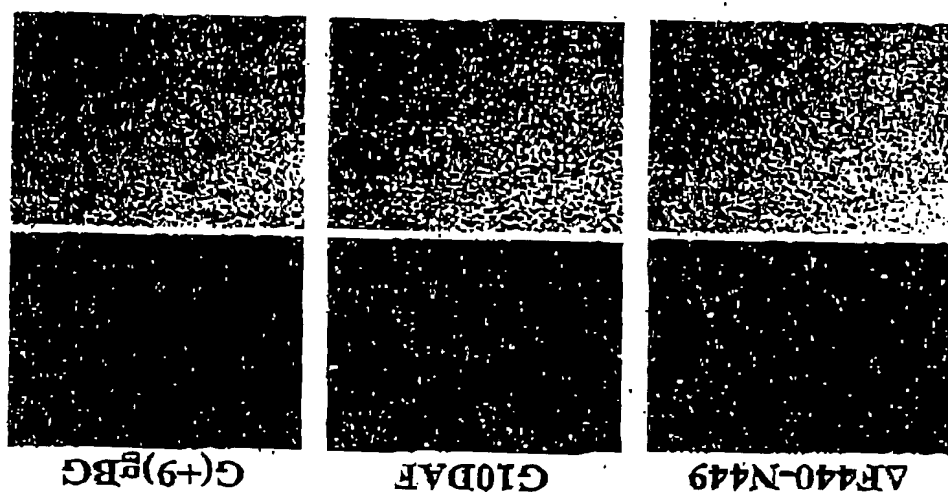


Figure 22

Figure 23



B



A

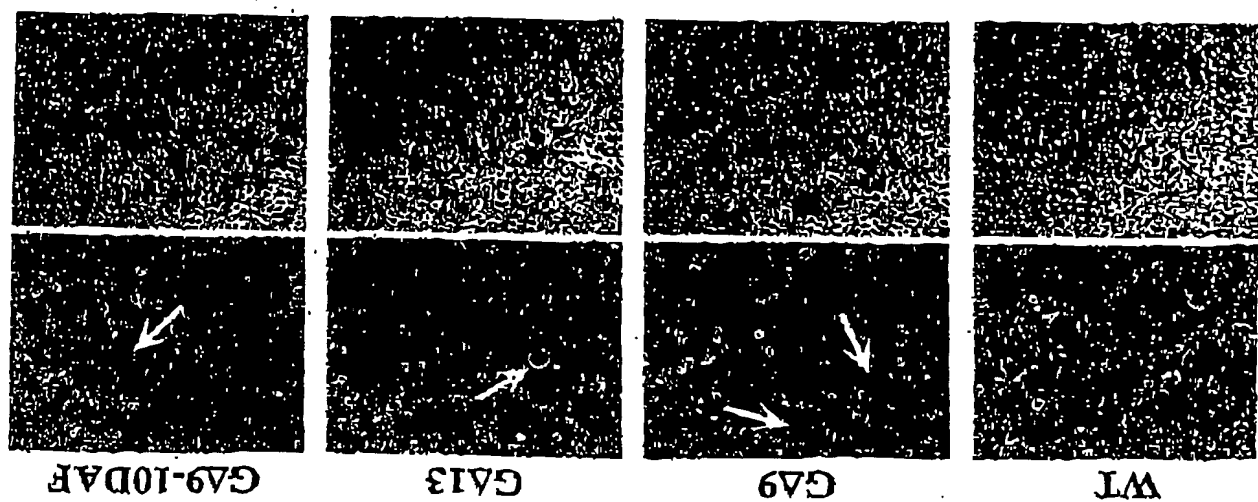


Figure 24



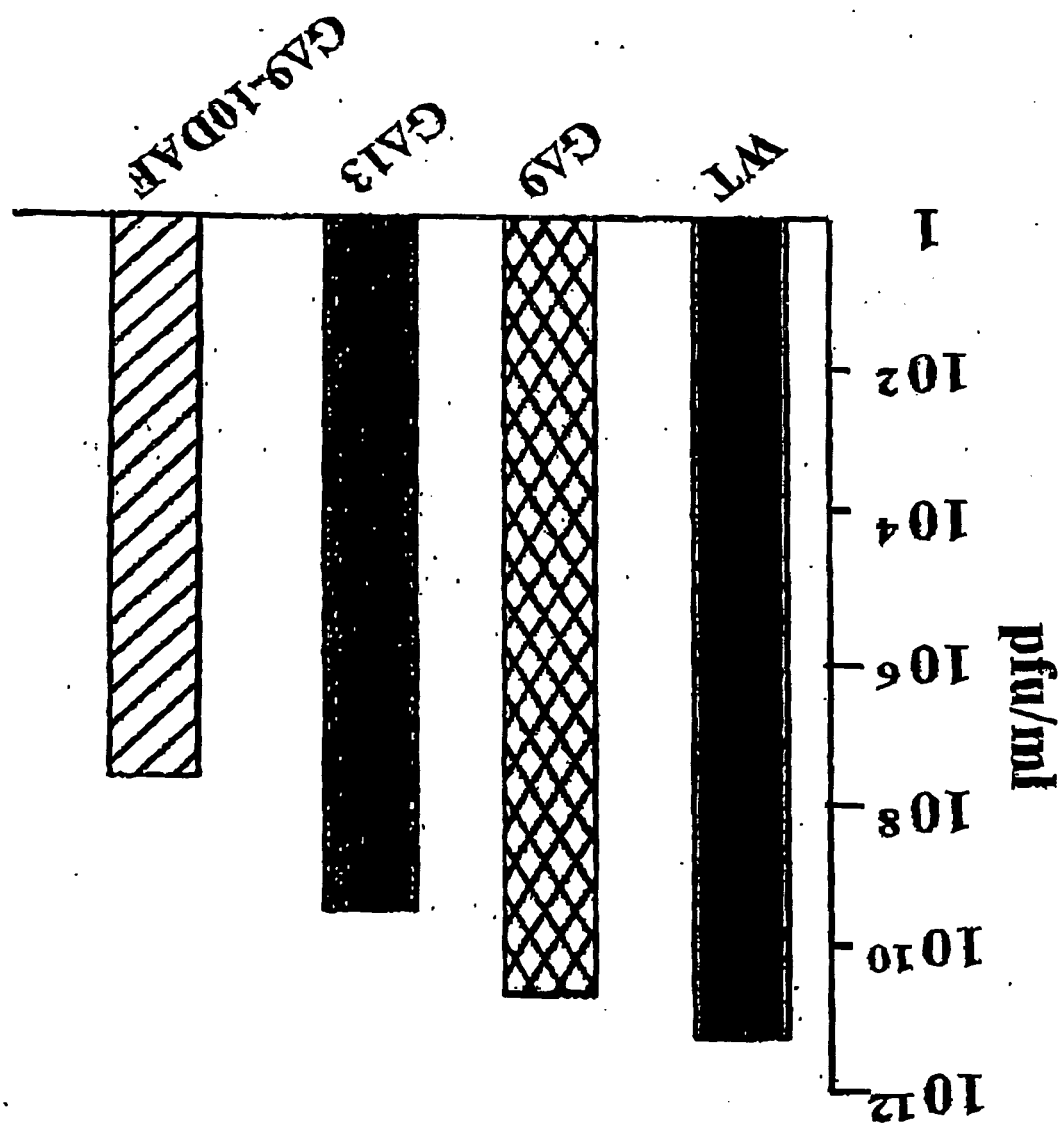


Figure 25

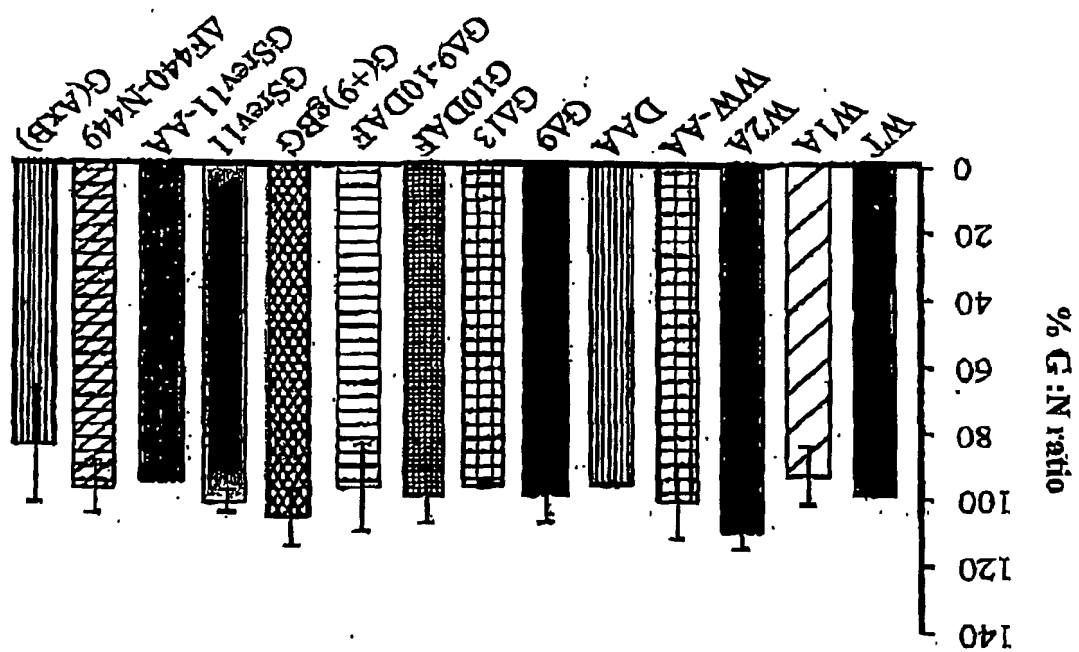


Figure 26

Figure 27

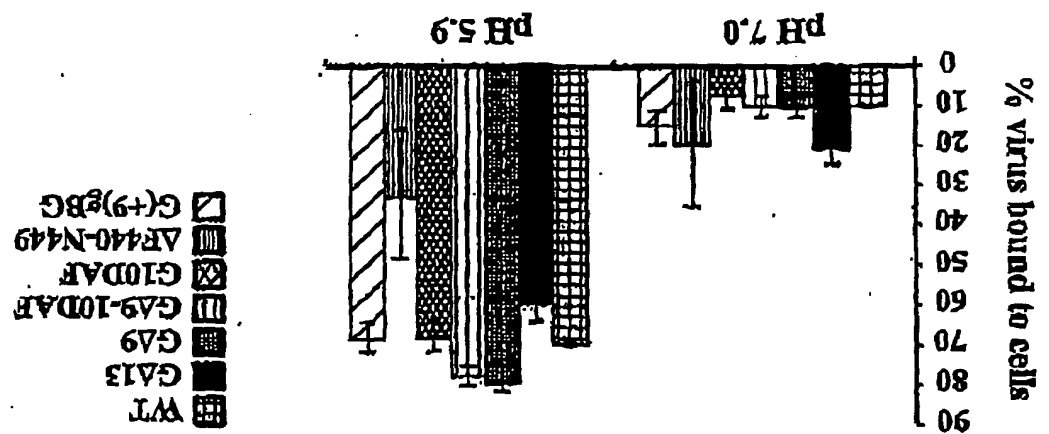
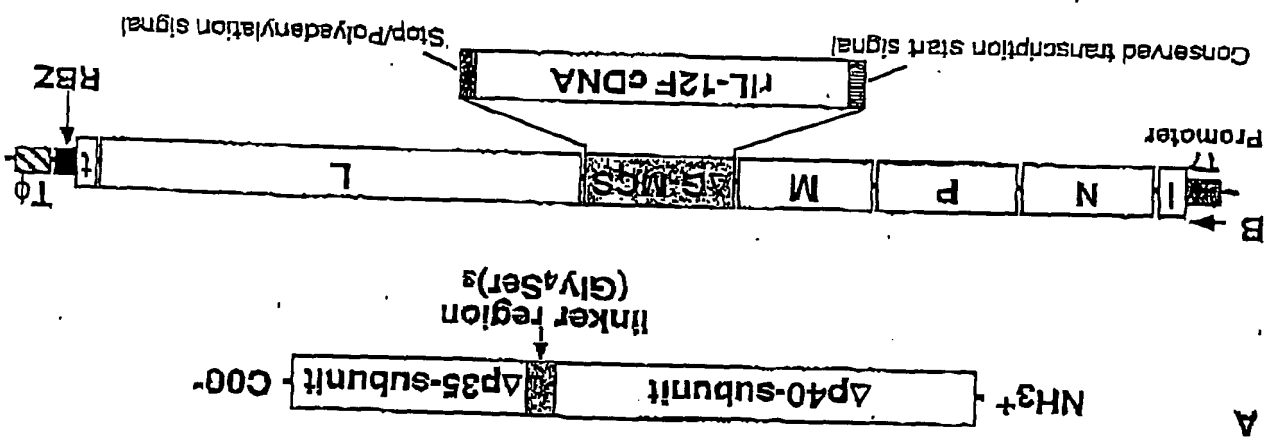


Figure 28



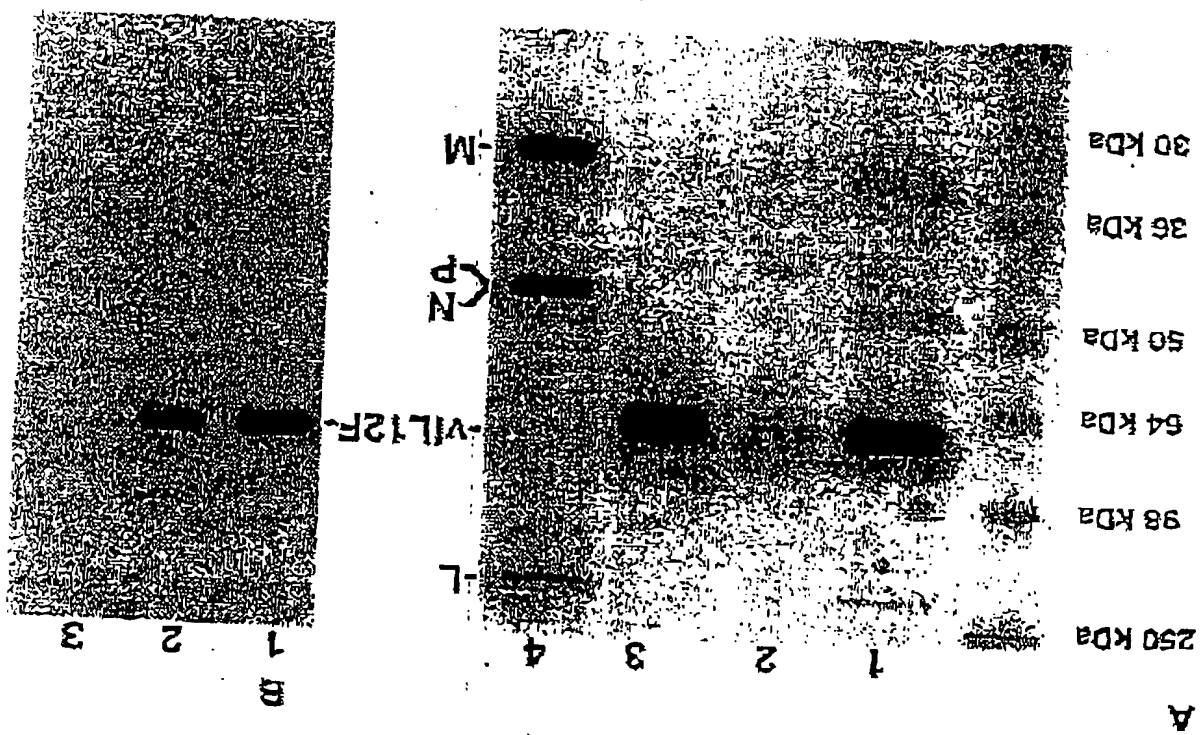


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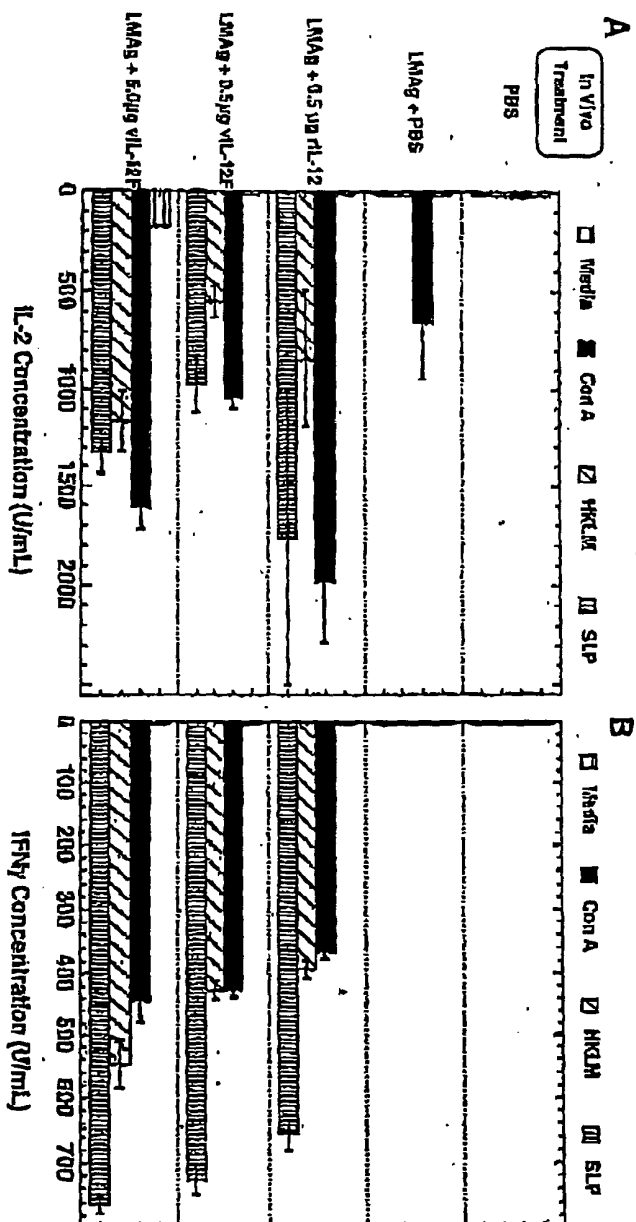


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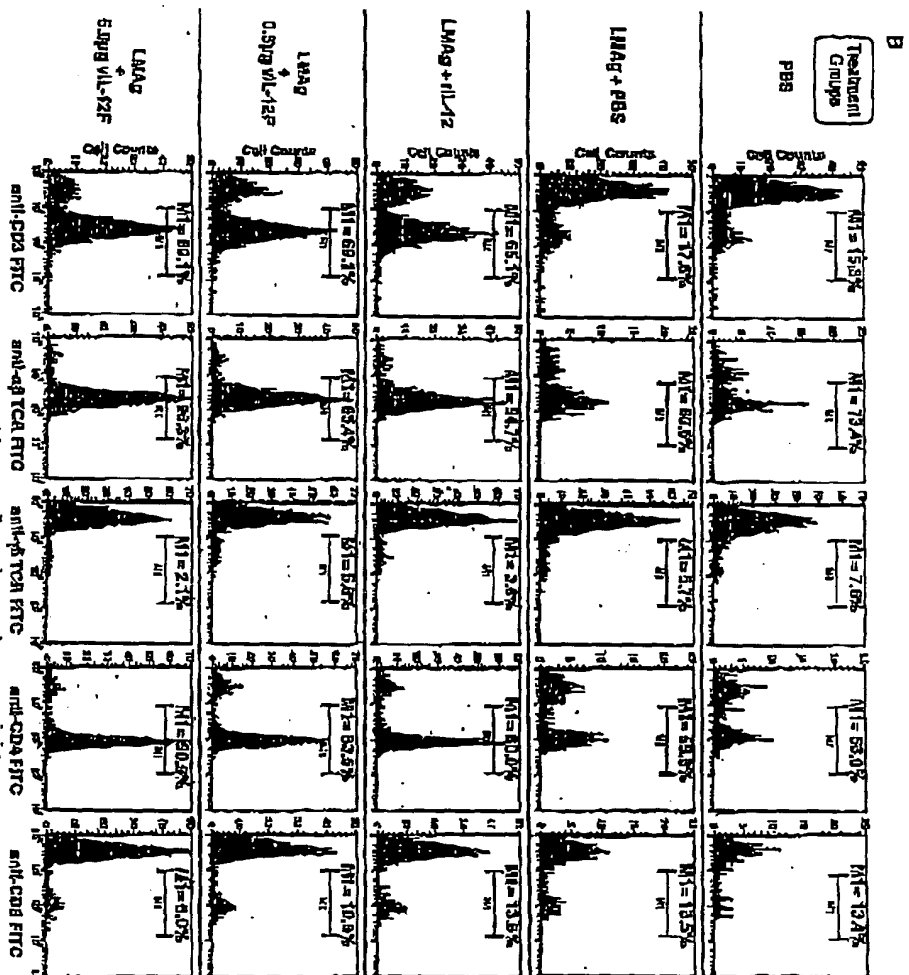


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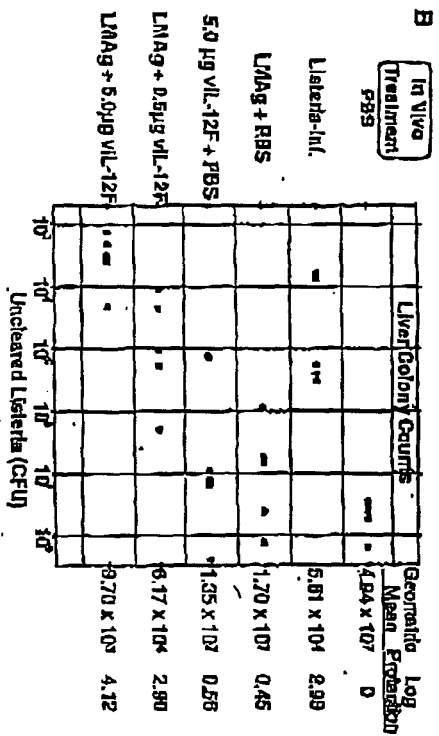
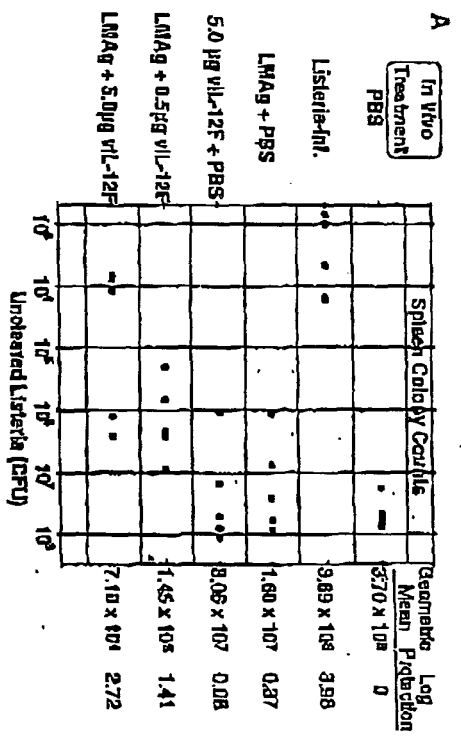


Figure 32



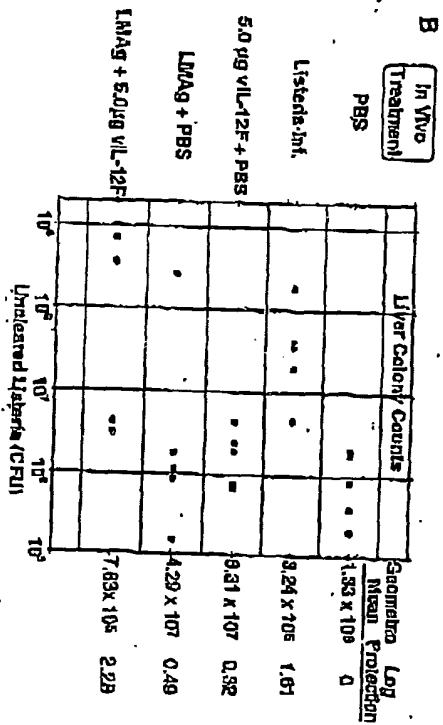
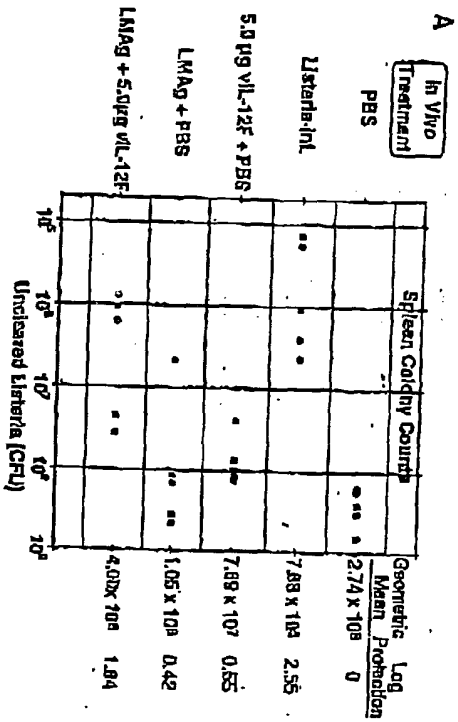
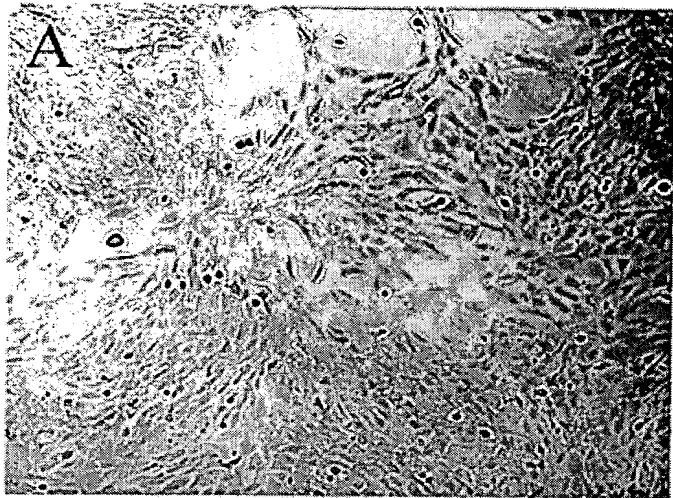
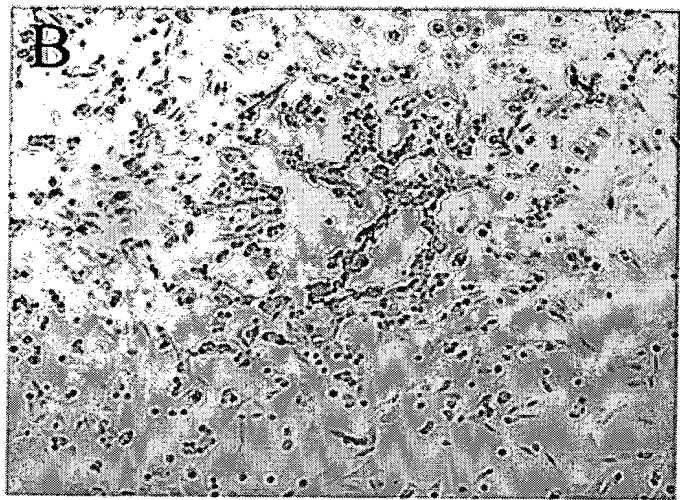


Figure 33

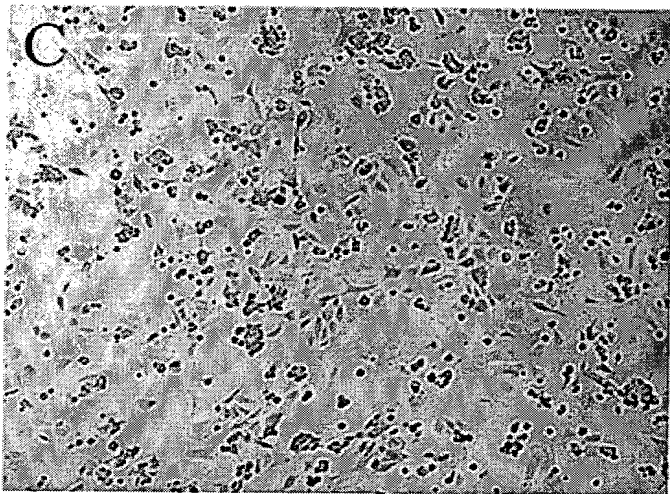
0 hr



10 hr



24 hr



48 hr

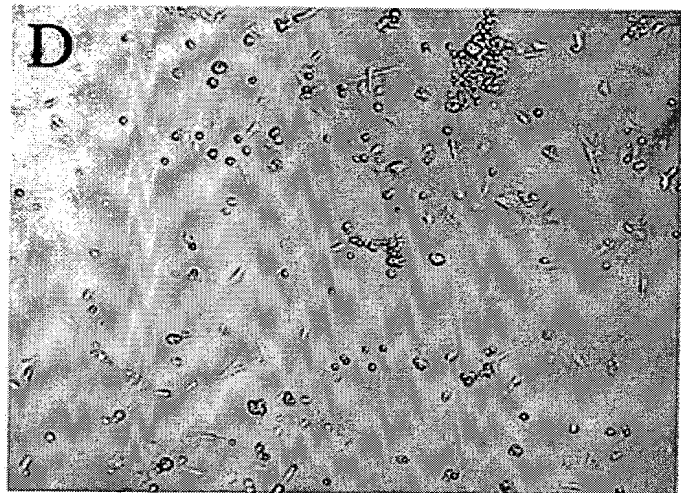


Figure 34

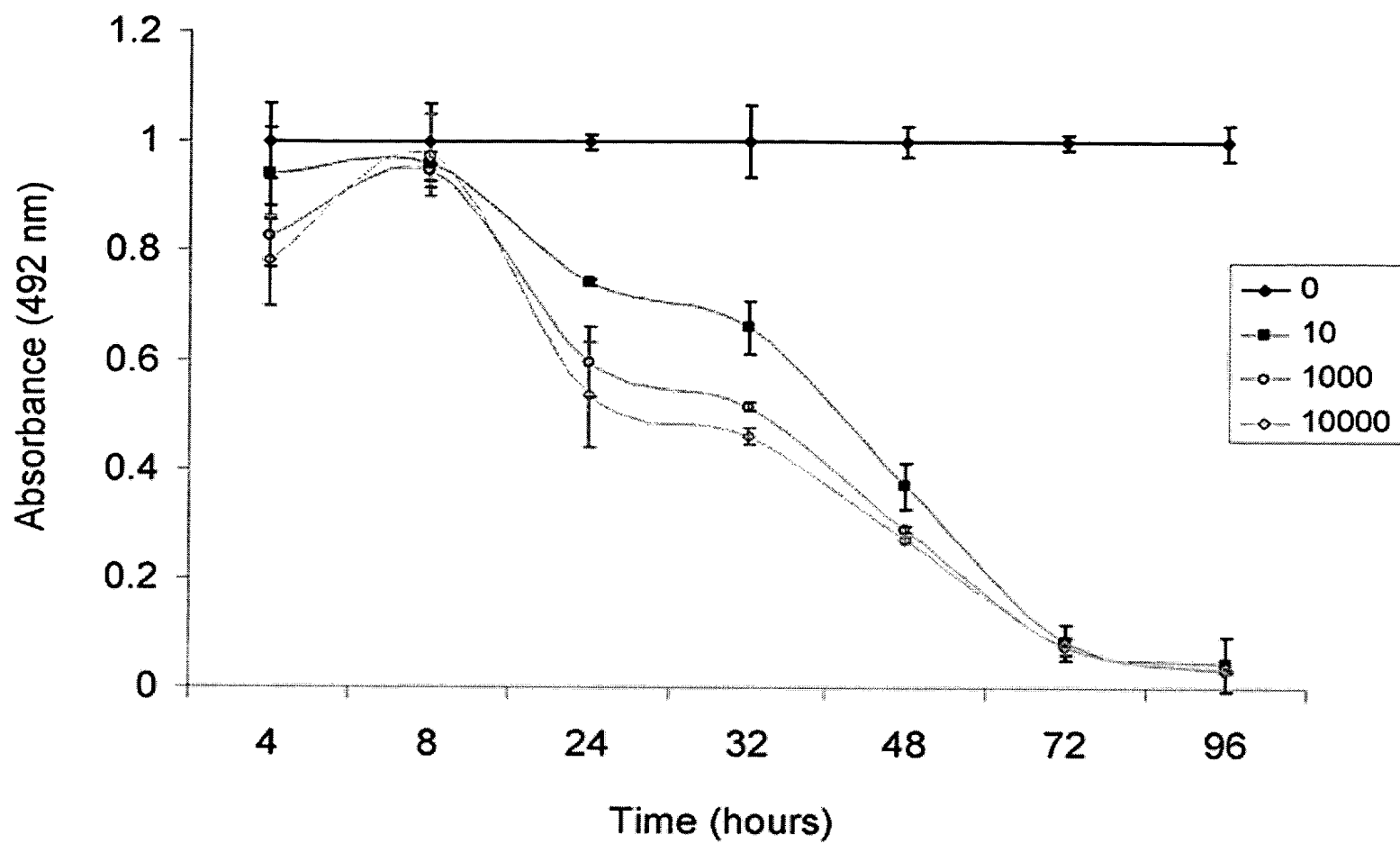
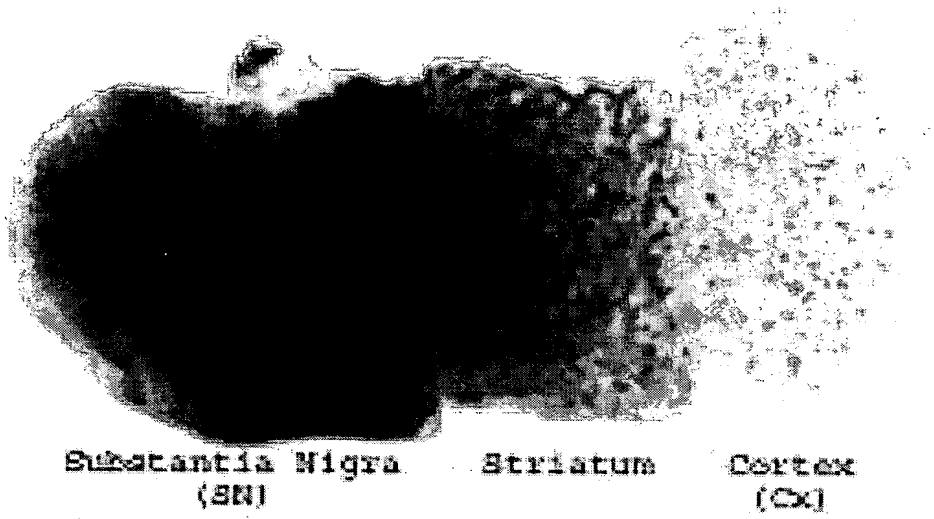
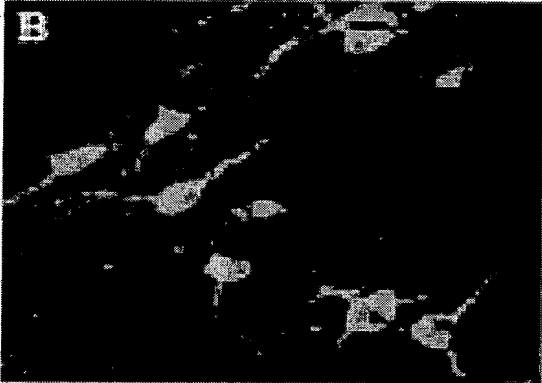


Figure 35

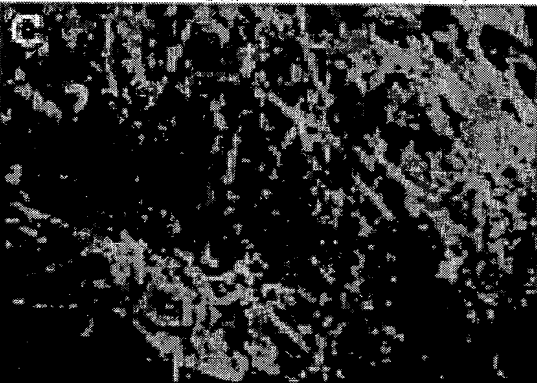
A



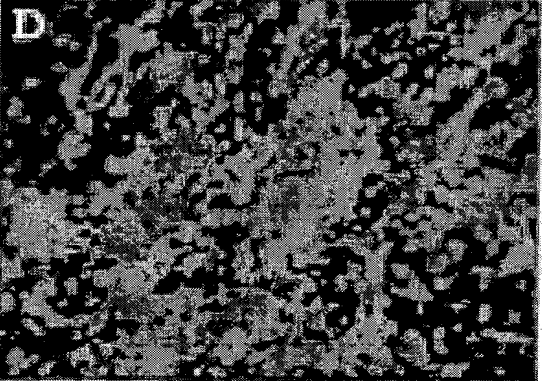
B



C



D



E

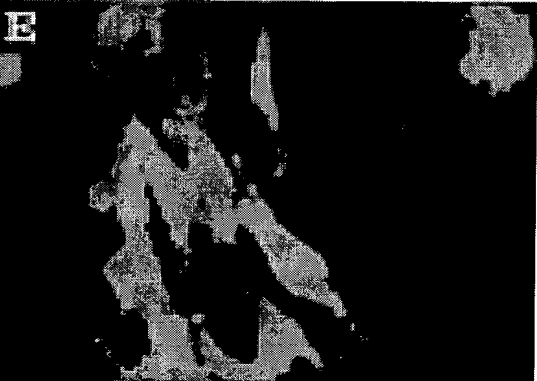


Figure 36

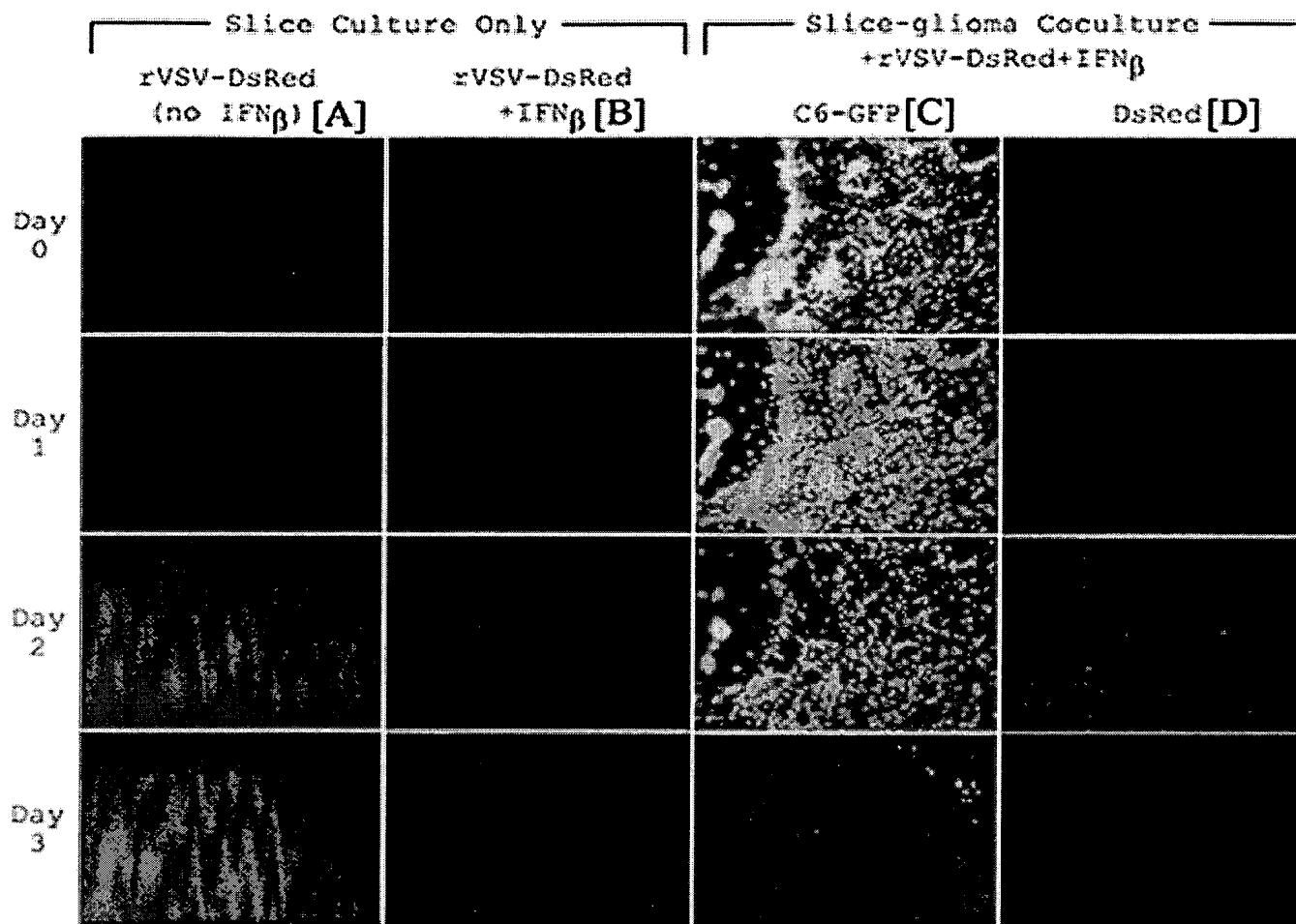
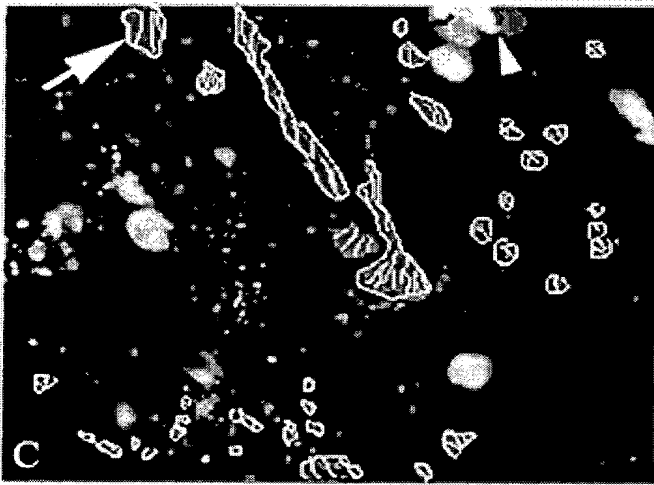
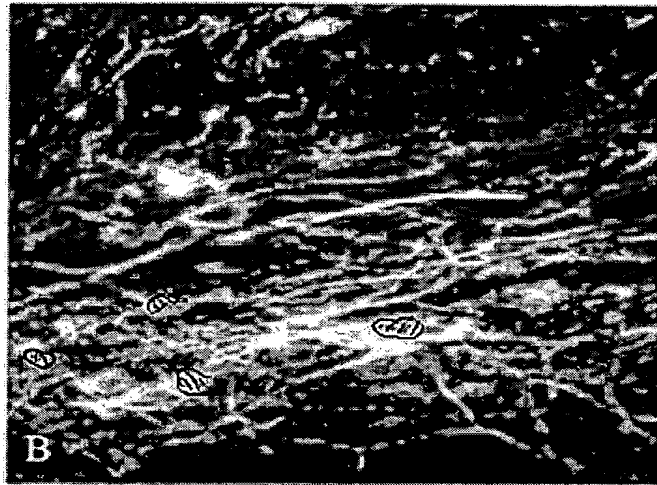


Figure 37

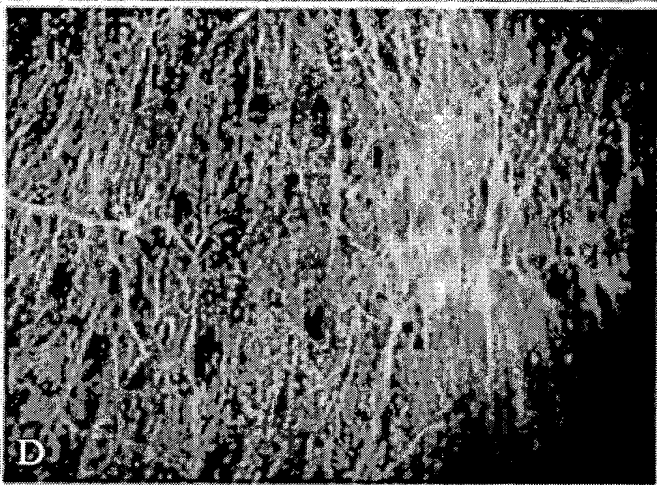
VSV-wt w/ no IFN



VSV-wt w/ 1,000u IFN



VSV-wt w/ 1,000u IFN on C6 co-cx



normal slice w/ IFN only

Figure 38

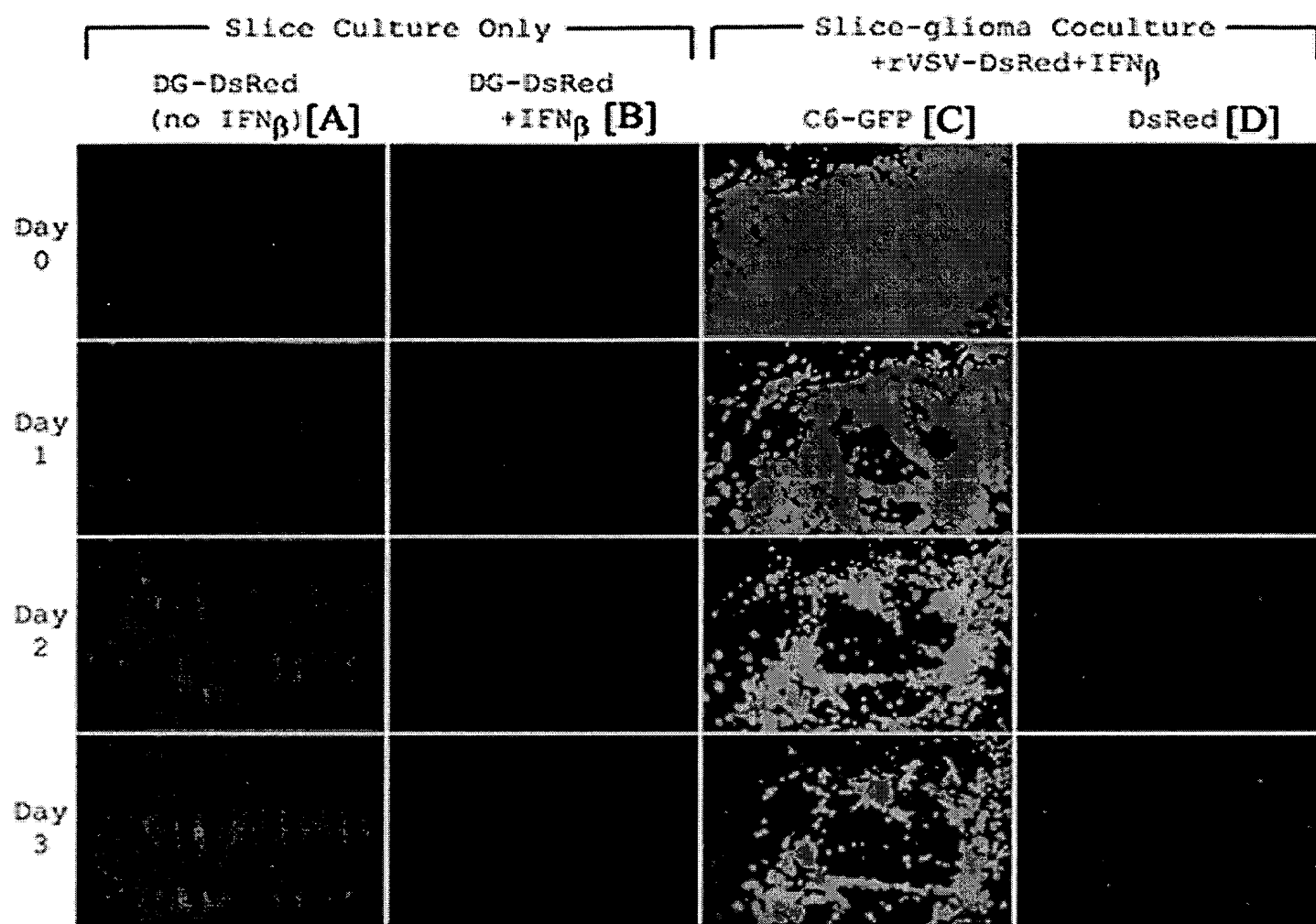
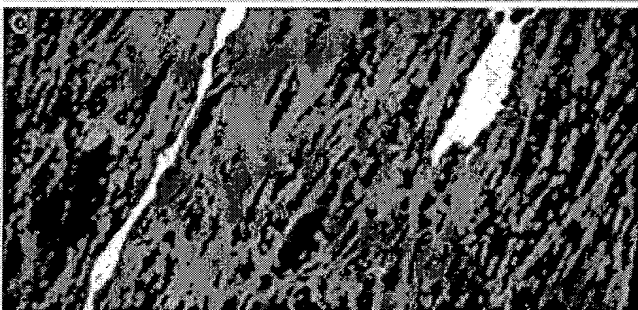
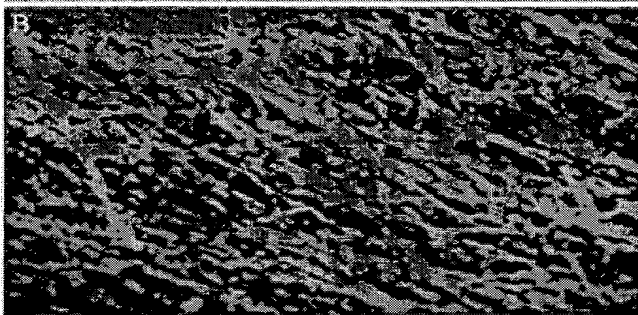
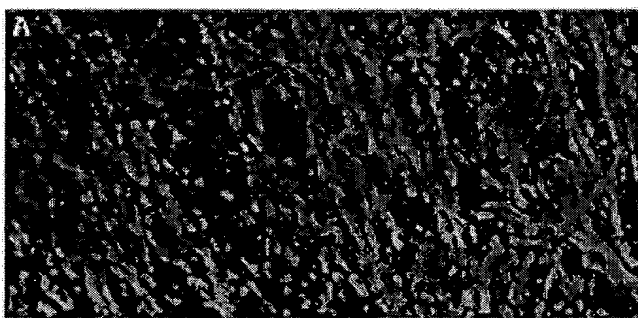


Figure 39

MAP2 Immunohistochemistry



Slice Culture  
Only

Slice-glioma  
coculture

Figure 40



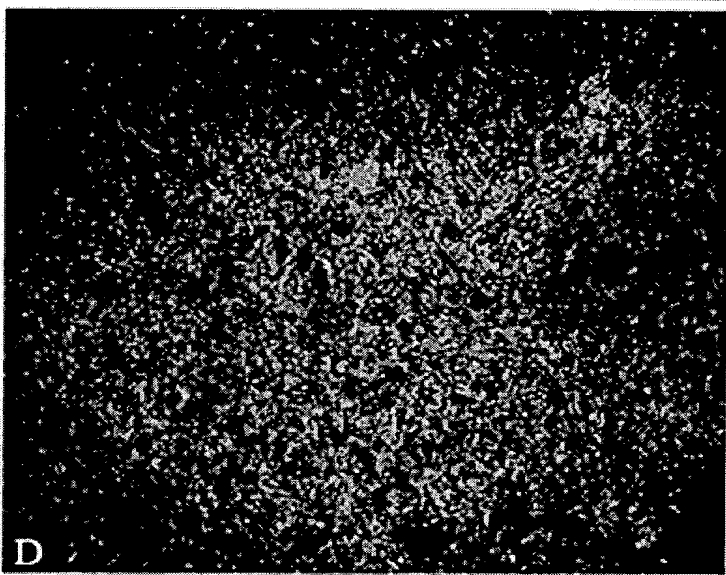
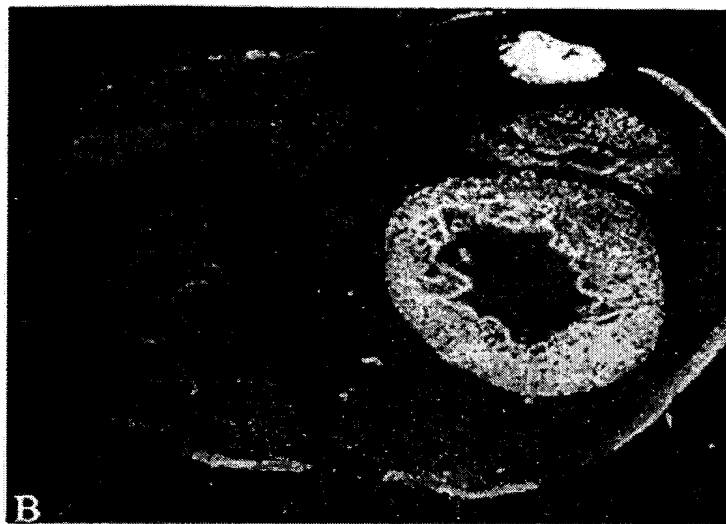
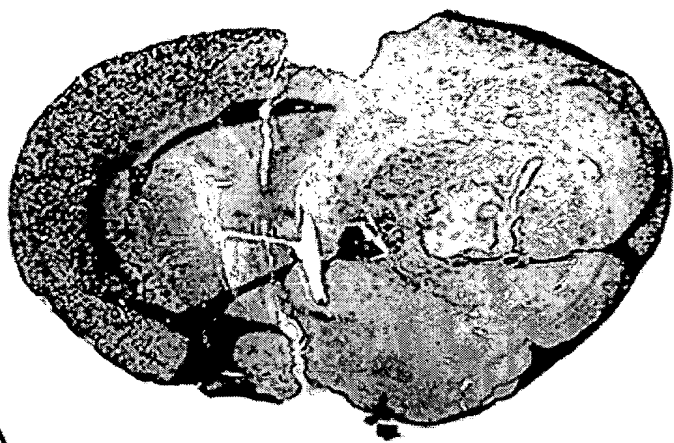


Figure 41

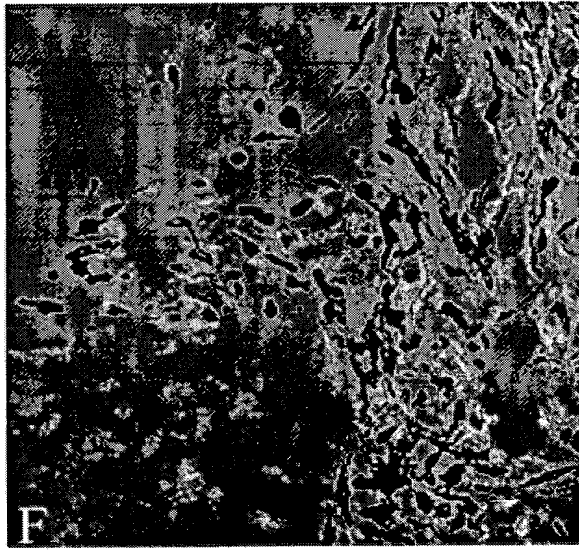
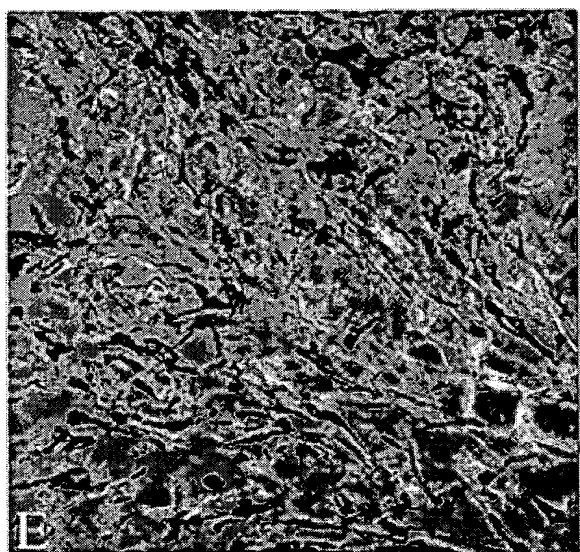
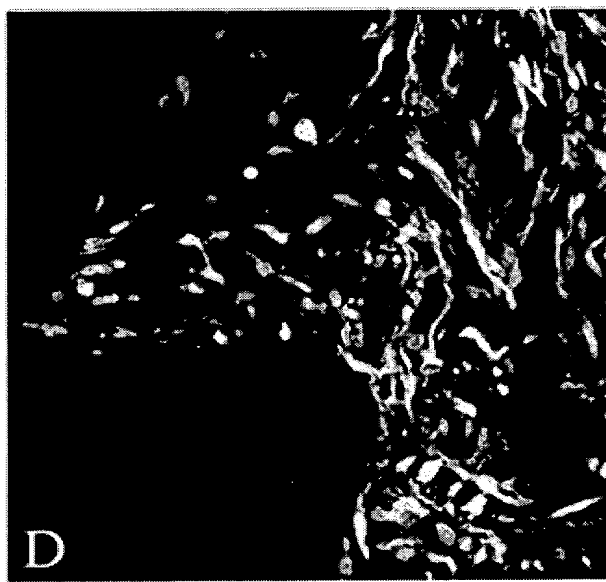
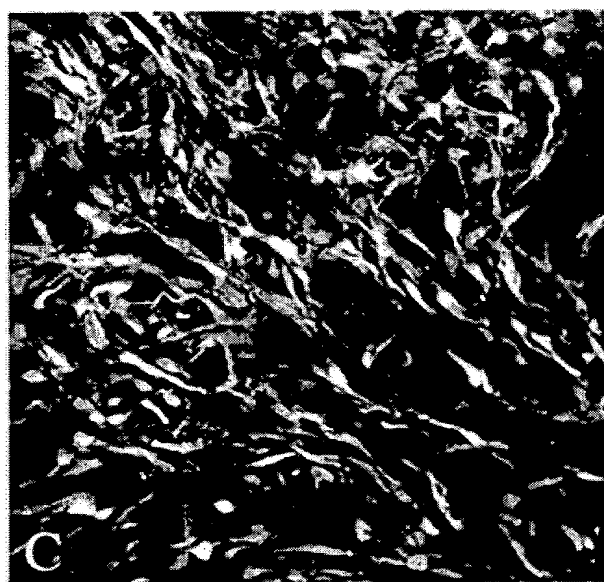
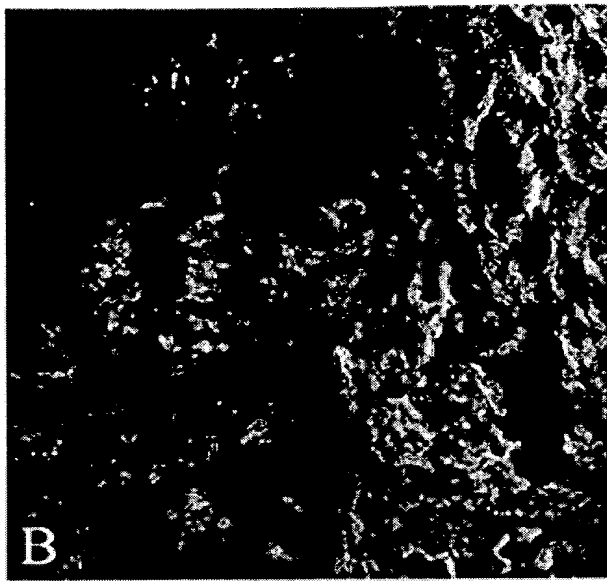
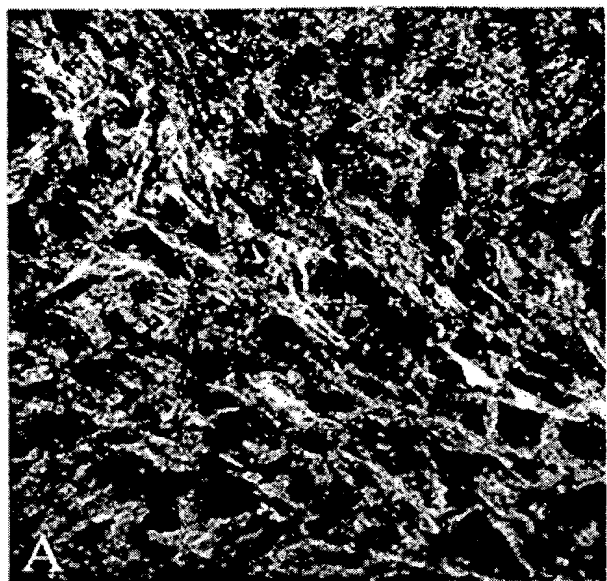


Figure 42